Biological Correlates of Human Male Reproductive Strategies and Behavior

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Abstract

Studies suggest that men's genotypes and endogenous hormones interact with their contextual environment to influence mating decisions and paternal behaviors. Participants, 20-45 year old men, responded to questionnaires and gave blood samples before and after romantic and paternal caregiving interactions. The romantic setting included viewing two video clips (with partner, if applicable). The paternal setting included caring for a RealCare baby doll. Questionnaire data included demographics, personal history, relationship status, etc. Hormonal data included enzyme immunoassay of testosterone (T), cortisol (CORT), oxytocin (OT), and vasopressin (AVP). Genotyping of receptor polymorphisms included sequencing for androgen receptor gene (*AR*-CAG), three single nucleotide polymorphisms (SNPs) of *OXTR*, one SNP for *CD38,* and two microsatellites for *AVPR1A*. Chapter 1 results support the hypothesized 3-Group model with men in the Bachelor Group reporting demographic data consistent with short-term mating strategies and higher baseline T levels than men in the other two groups. Men in the Provider Group exhibited a mix of short-term and long-term mating strategies, whereas men in the Direct Father Group generally exhibited long-term mating strategies. Chapter 2 results indicated that OT levels were higher in men that spent more time with children and men in the Provider group. Higher OT levels in the Provider group were specific to particular recessive homozygous genotypes: *OXTR* 2254298 (GG > AA/AG), *OXTR* 53576 (GG > AA/AG) and *CD38* (CC > AA/AC). Most interestingly, men in the Provider group experienced an increase in OT levels following the video session, but a decrease following the baby doll session. Chapter 3 results showed decreases in CORT levels during the baby doll session, and men in the low parent group of the RS/RS model

experienced greater increase in ratios of OT levels. Although CORT levels declined in the baby doll session, different groups of men varied in the extent of decrease relative to other hormones. These study results will help inform public education of parenting support and potential interventions in instances of pathology, such as paternal abuse or neglect.

Summary

This study examined the potential endocrine and genetic correlates of human male reproductive strategies in St. John's, Newfoundland, Canada. I hypothesized three distinct reproductive strategies in human males and compared them to the alternative hypothesis that partnership and parenting responses are physiologically separate and distinct. To test my hypotheses, I performed a laboratory study of reported partnership perspectives and physiological responses to romantic video stimuli, as well as, reported parenting perspectives and physiological responses to a programmable baby doll. I took blood samples from 52 men before and after experimental interaction to assay for the steroid hormones, testosterone and cortisol, along with the neuropeptides, oxytocin and vasopressin. Blood samples were also used to perform DNA sequencing for analyses of genetic markers for variation in the androgen receptor gene (number of CAG repeats), oxytocin receptor gene (*OXTR*-SNPs), and vasopressin receptor gene (*AVPR1A*-Micros). Analyses indicates that hormones and genetic markers are correlated with partnering and parenting in individual and context specific ways. For example, testosterone levels respond to the sociosexual context with one of three strategies, whereas oxytocin levels seem to respond to the sociosexual context through the bifurcated strategies of partnering

and parenting independently. Taken together, these results contribute to a complex picture of male reproductive physiology and behavior that explains the highly flexible and adaptive nature of human reproduction.

Keywords: Testosterone, Cortisol, Oxytocin, Vasopressin, *OXTR*, *AVPR1A*, *CD38*, *AR*-CAG Repeats, Reproductive Strategies, Pair-bonding, Paternal Behavior

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Introduction

by

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1. Introduction

1.1. Evolutionary Foundations

Human life histories differ from those of our closest relatives, the great apes, in that we exhibit exceptionally long periods of juvenility and high levels of fertility despite the high energetic costs per offspring (Walker et al., 2008), suggesting that additional caregivers are of significant value to the offspring's survival and development. Reproductive effort of males is inherently divided between paternal investment and mating effort (Clutton-Brock and Parker, 1992; Trivers, 1972), with the proportion allotted to each type of investment differing across human populations (Geary, 2000). Modern humans frequently have long-term male-female associations and varying amounts of paternal care (Quinlan and Quinlan, 2007). Additionally, the availability of allocare, such as paternal care, contributes to increased birth rates (Ross and MacLarnon, 2000) by decreasing the energetic demand of childcare for the mother. Within the possible permutations of allocare, it is high levels of paternal behavior that are associated with long-term pair-bonds (Broude, 1983).

When pair-bonds exist and paternal care is present, the factors influencing the amount of paternal care are of great interest to both academics and the general public, and this interest has resulted in a recent increase in studies involving romantic relationships and parenting behavior. These studies have often focused on either parenting or pair-bonding, but rarely both. In order to accurately represent the overlap between these topics, I will describe how each affects, and is affected by family dynamics, paternal behavior, and the underlying physiology of these processes. To set the foundation for this explanation, I will first address the evolution of pair-bonding and parenting.

When considering the pressures that shaped the evolution of parenting and pair-bonding, it is important to remember the key features of mammalian reproduction, namely internal fertilization, live birth of altricial young, lactation, and extended juvenility. The specific characteristics of mammalian reproduction that have selected for male participation in parenting and the maintenance of pair-bonds are much debated. Marlowe (2000) evaluated the potential evolutionary sources of human paternal care and concluded that paternal care can be driven by the need to provide offspring care and protection or for the purposes of mate guarding and continued sexual access. Additionally, a recent review of human mating system studies suggests that while there is considerable variation, the most common strategy across cultures seems to be serial monogamy (Schacht and Kramer, 2019). To put evolutionary history and modern life practice into perspective, it is also important to consider the evolutionary history that may have been at play during the selection of mating behaviors as well as the current availability of birth control, which allows for greater selection of reproductive events (Nargund, 2009). This modern scientific advancement may be adding flexibility to an already plastic system. Taken together, these studies indicate that there are multiple sources of individual variation, both in the evolution of long-standing mating strategies, as well as, modern expressions of evolutionarily selected behaviors.

Other intuitively appealing explanations have been proposed for the evolution of pair-bonding. One such explanation for the evolution of paternal behavior is the "mate guarding hypothesis" (Palombit, 1999) which states that mate guarding is the inspiration for pair-bonding [and therefore paternal behavior]. In this explanation, mother –infant bonding evolved prior to the pair-bond. However, others argue that it was not mate

guarding that drove selection of pair-bonding, but that pair-bonding evolved out of the necessity to provide for the mother as she provided extended infant care (as measured by duration of breast-feeding). Having a parenting partner may allow the mother to breast-feed longer by providing additional resources to the mother and/or by reducing the physical demands of infant care (Quinlan and Quinlan, 2008). Alternatively, allocare can assist in the weaning process by distancing the infant from the mother and mediating the "on demand" feeding that extends interbirth intervals (Fouts et al., 2005). I interpret these findings to mean that cultural differences in average breastfeeding duration (i.e., shorter than the world-wide average in North American and European countries) are differentially affected by the presence of a parenting partner, in addition to the obvious geographical differences in access to food resources.

The dichotomy between pair-bonding and parenting is further complicated by the division of paternal behavior into direct and indirect male caregiving, which are both relevant in human evolution. Direct care can include activities such as carrying, feeding, and grooming the infant; while indirect care can include provisioning mother and/or offspring, familial protection, and resource defense (Kleiman and Malcolm, 1970). The allocation of energy toward these activities directly affects a male's ability to pursue other mates or produce more offspring. With the advent of judicial management of post-divorce custody and financial obligations, are male choices related to parenting different than they otherwise would be? Is remarriage with another spouse more appealing for males because of opportunities to produce more offspring? Do males employ various reproductive strategies throughout their lifecycle? Or, are choices based on the current local context and/or evolutionarily driven predispositions?

While choices about paternal care and/or provisioning are certainly influenced by the local context, the overall trend over the past few decades is that [N. American] fathers have been increasingly involved with their children, which may affect the quality of the relationship and attachment that men have with their children (Maurer et al., 2013; Pleck, 2007). The expression of paternal behavior can include holding, feeding/caring, playing with the infant, or caring for older siblings so that the mother is free to care for the infant (Winking, 2010). For the purposes of this study, I will be focusing on behaviors that characterize primary caregiving, such as feeding, changing, and rocking. I chose "caregiving" behaviors in lieu of "play" behaviors as I want to focus on primary parenting rather than behaviors that support the mother as the primary parent. To do this, I chose to use the RealCare baby doll which allows for consistent simulation of caregiving condition, as in a previous study of nurturance and testosterone in men (van Anders et al., 2012b).

Consistent with the idea that paternal certainty is a significant predictor of offspring survival and fitness, men from rural Senegal invested more in children who resembled them (Alvergne et al., 2009). The fathers' level of paternal investment was correlated with markers of relatedness, and the children who looked and smelled more like their fathers had better health and nutrition than children showing less resemblance. Similarly, a study of North American men found that paternal investment was highest with greater resemblance, perceived mate value, perceived fidelity, and more exclusive relationship status, with declines in investment occurring when the relationship value was reduced (Apicella and Marlowe, 2007, 2004). Taken together, these data suggest that complex factors, such as paternity certainty, are involved in the decision to provide

paternal care and the decision to maintain a high level of care over time.

The various characteristics relevant to pair-bonding and parenting choices are heavily influenced by the underlying physiological processes, including brain functions, which may contribute to individual differences. For instance, neurons involved in the release of oxytocin (OT) share interconnections with mesolimbic dopaminergic neurons, serving both romantic and parental attachment through sexual behavior and sexual preferences (Melis and Argiolas, 2011). Given the evolutionary age of parent-offspring bonds, pair-bonds may have evolved from the neuroendocrine basis of maternal nurturance (Fernandez-Duque et al., 2009). The connection between brain function, sexual behavior, and parenting demonstrate the necessity of examining both the relationship between the man and his infant, as well as the man and his partner. These studies will be, to my knowledge, the first to experimentally compare pair-bonding and parenting strategies in humans (for review of non-human primate species, see {Formatting Citation}.

The importance of understanding the evolutionary foundations of pair-bonding and parenting is clearly evident when considering the generational effects of parenting practices, which reach far beyond infancy. Not only is responsive and caring parental care essential to the development of the infant, but also to the development of that infant's ability to form secure attachments in adulthood, including pair-bonding (Atzil et al., 2011; Sroufe, 2005). The establishment of secure adult attachment is important as it has been shown that adult attachment predicts maternal brain and oxytocin response to infant cues (Strathearn et al., 2011), which then allows for sensitive parenting of the next generation.

This study will examine the possible distinctions between three reproductive strategies: Bachelor, Provider, and Direct Father. In the case of the 'Bachelor', males would reduce long-term investment by taking the 'high-quantity, low-quality offspring' approach. The 'Provider' would best fit the description of 'mate guarding' as the male would invest in the offspring as much as necessary to maintain access to the mating partner. Finally, the Direct Father would assure their continued mate access, as well as their paternity certainty, by spending a significant amount of time with the female and/or infant but the focus would be on enhancing the development or survival of the infant. The results of this study may suggest positive steps toward the improvement of pair-bonding and paternal interactions.

1.2. Steroid Hormones

Interest in the implied trade-offs between the male reproductive strategies of mating and parenting effort have led to the study of hormonal shifts in a variety of mammalian and avian species (Storey et al., 2000; Wingfield et al., 1990). A prime example of this interest is the multitude of studies involving measures of the testosterone (T), which is an ideal candidate for such research as it has been implicated as a possible marker of varied reproductive strategies and associated with a variety of sociosexual behaviors. Higher T levels are associated with increases in somatic muscle mass, aggression, libido, and sexual stimulation (Bribiescas, 2011), as well as self-protective tendencies such as decreased trust in women (Bos et al., 2010) and increased trust in men (Bird et al., 2017), as well as in-group favoritism (Reimers and Diekhof, 2015).

When considering the whole of a man's reproductive context, care must be taken to differentiate between pair-bonding and parenting as high T levels are linked positively

to sexuality, but elevated T is generally negatively linked to nurturance (reviewed in Storey et al., 2020). Ultimately, sexual intimacy pathways likely take priority when both sexual and nurturant pathways are activated (van Anders et al., 2012a). For instance in women, anticipation of sex was found to increase T, but not cuddling in and of itself (van Anders et al., 2007). Comparable data regarding men's T response to cuddling is as yet unavailable. However, taken together, these findings suggest that studies of paternal behavior must address the presence or absence of the mother and her potential influence.

Hormonal responses reflect a bi-directional interaction between the individual's internal state and the external environment (Edelstein et al., 2011; van Anders and Goldey, 2010). Men's relationship orientation, preference for a long-term monogamous partner or interest in multiple partners predict their T levels along with the frequency of sex with the relationship partner (van Anders and Goldey, 2010) since increased sexual frequency within the pair-bond is associated with lower T levels. In addition, men with higher T levels are less likely to marry, more likely to divorce, and more likely to have a lower quality marriage (Booth and Dabbs, 1993), and so are less likely to put themselves in the nurturant situations that would reduce their T levels. Moreover, the pattern of reduced T in pair-bonded relationships has not been consistent over all contexts and cultures (Gray, 2003).

With regard to situations specific to parenting, it has been shown in a variety of contexts that increased contact with an infant is associated with lower T levels in men (Gettler et al., 2012; Kuzawa et al., 2009). For instance, Muller et al. (2009) found that increased parental investment, rather than parental status or the presence of a pair-bond, predicted lower T levels in men. Additionally, Storey et al. (2011) found that a father's T

levels were affected by recent child contact, and two types of men were identified, those that reacted differently on days of high and low toddler contact, and those that reacted similarly on both. Men who were more attentive to the child and had been away from their child longer had the largest decline in T. The distinction between these two types of men was part of the founding concepts for the Provider and Direct Father groups as both of those groups of men are characterized by varying levels of paternal investment. Men in the Provider group would be those individuals that prioritize investment in the family in the form of provisioning and protection, whereas men in the Direct Father groups would be those men that prefer to take a primary role in childcare and home making (i.e., either equal to or greater than the role of the mother). It is important to note that this study will also be including men that fit in the Bachelor group, which is characterized by little to no interest in parenting or even partnering. The Bachelor group would not have been represented in the study by Storey et al. (2011), as their participants only included actively investing fathers, and were likely subject to a selection bias of highly paternal males.

The quality of paternal investment seems to be another contributing factor in T level responses, with responses to baby cries where no care is possible producing increased T levels relative to a context where successful care was possible (van Anders et al., 2012b) (see also van Anders et al., 2014). This outcome could be a possible explanation for the conditional response of T when in the presence of the mother, as she controls the level of participation that he can provide. On the other hand, it is also possible that the father only mounts the appropriate biological caregiving response when necessary. Lucassen et al. (2011) suggest that studies should examine whether the father has a more direct or

buffering effect on child development. A direct effect can be thought of as anything done by the father that directly contributes to the child's fitness, such as teaching them useful life skills. A buffering effect is an effort by the father that increases the child's fitness indirectly, such as supporting the family financially.

Another important steroid hormone involved in male behavior is Cortisol (CORT), which is often examined in relation to stressful experiences and response. For example, men experience elevated CORT levels during stressful situations (Kirschbaum et al., 1995, reviewed in Erickson et al., 2003), as well as in situations of more positive arousal such as the early phases of romantic relationships (Marazziti and Canale, 2004), and just before the birth of their babies (Berg and Wynne-Edwards, 2001; Storey et al., 2000). Results are mixed for the relationship between paternal investment and CORT levels: greater paternal involvement has been linked to both higher (Kuo et al., 2018), lower (Bos et al., 2018), and declining CORT levels (Gettler et al., 2011b). This complex pattern of results highlights the importance of examining CORT responses in specific contexts. Introduction of CORT and examination of its potential interactions with the other study hormones will be presented in Chapter 3.

1.3. Neuropeptide Hormones

Oxytocin (OT) and arginine vasopressin (AVP) are nonapeptides, differing by only 2 amino acids. As they are relatively large molecules, and lipid insoluble, they do not cross the blood brain barrier easily. The production of OT and AVP is controlled by the hypothalamus, but they are released by the pituitary gland. The primary function of OT is smooth muscle contractions, including the contractions involved in parturition and milk ejection. AVP is primarily related to fluid balance and cardiac function. In addition to

their primary physiological functions, both OT and AVP are related to a variety of social behaviors (See Figure 1 and 2).

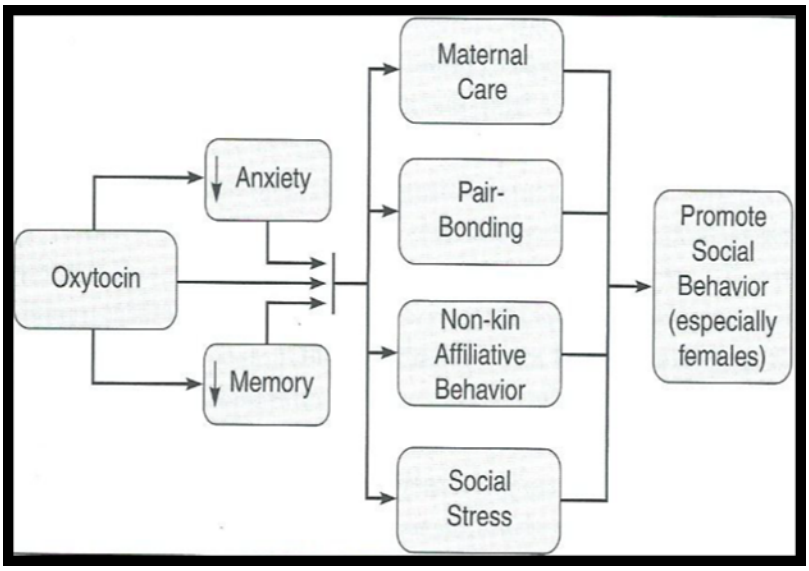

Figure 1. Behavioral effects of OT in humans (Grey & Ellison, 2012).

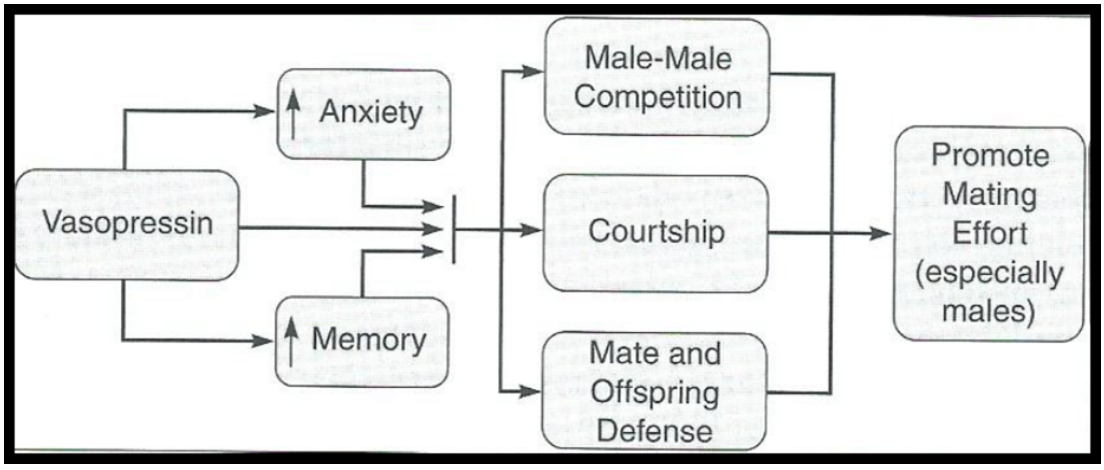

Figure 2. Behavioral effects of AVP in humans (Grey & Ellison, 2012).

As shown in Figures 1 and 2, correlational studies have assessed the relationship between OT, AVP, and human social behaviors. These studies used peripheral measures of the hormones, despite a long standing controversy about whether peripheral levels of OT and AVP were representative of central levels (Ebstein et al., 2012; Carson et al., 2014, 2015) or not (Kagerbauer et al., 2013; McCullough et al., 2013; Valstad et al., 2017). The evidence that peripheral levels are reflective of central activity is important to the study of human behavior as it allows for non-invasive measurement of hormone levels (Feldman et al., 2013), such as the techniques used in this study.

Early childhood experience has a significant effect on the development of social behaviors and their biological foundations. For instance, lower levels of OT and/or AVP have been found in children who had experienced early childhood neglect (Fries et al., 2005) or in women with a history of abuse (Heim et al., 2009). Additionally, OT levels increased in family-reared children following maternal interaction, whereas neglected children did not experience such a change, suggesting that sensitive parenting contributes to a well-developed OT/AVP system. Similarly, fathers given exogenous OT and recorded while interacting with their infants showed higher affect synchrony and play (Gordon et al., 2010, Feldman et al., 2010), resulting in increased OT levels of the infant as well. Higher levels of paternal sensitivity were also associated with more infant-father attachment security (Lucassen et al., 2011), pointing to the importance of children receiving high quality early care so that normal development of the OT/AVP systems is possible. Taken together, these data, and the results of genetic studies (see Feldman et al., 2012) indicate both a behavioral/social and genetic mode of OT transmission in humans.

The creation and maintenance of social relationships is complex and contextually

sensitive, and it appears that OT contributes to social bonding through its anxiolytic properties and anti-stress effects (Neumann, 2008). Intranasal administration of OT was shown to decrease men's approach distance to an attractive female in only monogamously attached males (Scheele et al., 2012), highlighting the importance of contextual cues in OT responses. Other data suggest that plasma and salivary OT were positively correlated with secure attachment to romantic partners (Atzil et al., 2011). Similarly, partners receiving intranasal OT showed increased proportion of positive relative to negative communications (Ditzen et al., 2009), and increased affiliation and emotional support (Gonzaga et al., 2006). Unfortunately, similar examination of AVP is lacking. However, AVP has been shown to be elevated in men experiencing relationship distress (Taylor et al., 2010), which is similar to studies of increased OT levels during periods of social bond stress (van Anders et al., 2011). Moreover, OT and AVP were found to be involved in couple communication and health outcomes, such as faster wound healing in couples with more positive communication and higher OT (Gouin et al., 2010). Cumulatively, these data support the importance of the effects of pair-bonding status when considering paternal behavior and its physiology.

This study will compare reactions of male participants observing romantic videos with their partners and those of male participants interacting with a programmable baby doll by examining the hormone levels prior to and following these two separate interactions. Please note that the use of this type of programmable baby doll is still new to this research area. Further validation of this technology and its effect on male physiology and behavior is warranted.

1.4. Genetics

To address individual variation at the molecular levels, studies of human phenotypes have examined correlations between genotypes and pair-binding and parental behaviors. For example, studies examining oxytocin receptor gene (*OXTR*) polymorphisms in mothers to (Bakermans-Kranenburg and van Ijzendoorn, 2008) and both parents (Feldman et al., 2012) found that specific allele combinations were related to higher or lower sensitivity of parenting. Additionally, it has been shown that specific *OXTR* alleles are associated with plasma OT levels (inverse relationship between risk and plasma OT, Feldman et al., 2012), affectionate touch behaviors (Weisman et al., 2012), and memories of childhood parental care (Atzil et al., 2011). The variation in the role of specific *OXTR* alleles and the effects of early social experiences on OT levels and social responsiveness highlight the complexity of these systems.

With the breadth of studies examining OT levels and alleles relative to human phenotypes, it is surprising that so few studies have examined similar effects with AVP. Of the few studies to date, notable results suggest that *AVPR1A* is significantly important in the reproductive and parenting behaviors of men. For example, a study found that *AVPR1A* rs3 334 bp allele was associated with low success pair-bonding, perceived marital problems, marital status, and marital quality as perceived by their spouses (Walum et al., 2008). As such, the combination of hormone measurement and gene studies provides an ideal platform for understanding their interaction.

1.5. Integrative Approach

Review of acute effects of steroid and peptides hormones on human socioemotional behavior suggests the potential for further examination of the links within

mating strategies. Current research suggests that extremes in CORT levels (i.e., high vs. low), as well as moderate T and OT levels and can facilitate social interactions and increase affiliative behaviors (Bedgood et al., 2014; Ponzi et al., 2016; Zilioli et al., 2015), while T may mediate AVP and can influence male behavior to increase motivation to act in challenge situations (Delville et al., 1996). However, further study is necessary to clearly understand the complex relationship between these hormones as well as other possible connections between them (i.e., T effects on OT).

Plasticity in peptide function induced by sex steroids is a potential mechanism for understanding individual differences in social behavior. The interaction of steroids and peptides has been demonstrated in partner bonding and paternal behavior. Specifically, the release of T and AVP during mating has been shown to contribute to pair formation (Insel and Young, 2001). One method of studying such a complex biological system is to partition social behaviors into nurturant behaviors and sexual behaviors, such as in the van Anders' Steroid/Peptide theory (van Anders et al., 2011; Figure 3). The S/P Theory is able to explain seemingly contradictory results including T, OT and AVP. Of specific interest is the identification of the two physiological systems: a nurturant system (evolved to support parent-infant bonds and infant survival) and a sexual system (evolved to support reproduction). This paper suggests that paternal behavior is directed by the individual balance between the opposing goals of mate access or direct infant care.

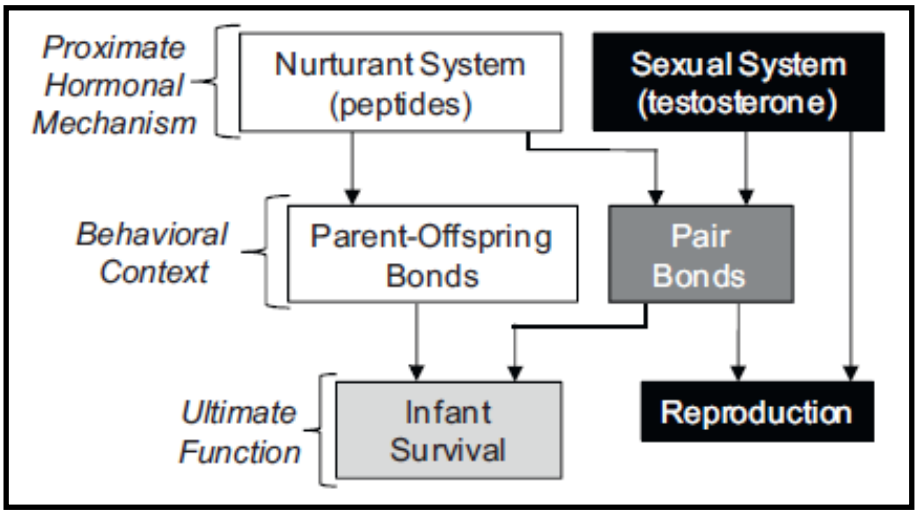

Figure 3. Steroid/Peptide Theory (van Anders et al., 2011).

Generally speaking, genomic effects represent long-term life strategies, some of which can be modified early in life under the influence of social variables (e.g., Champagne, 2008). For example, receptor types are genetically determined but the number of receptors may be affected by early social experiences. Non-genomic and indirect genomic effects, for example, hormonal responses to social stimuli, allow the individual to respond rapidly and flexibly to their local environment (see summary in Figure 4). The local environment can sometimes exert enough influence over time that genomic expression may be altered (i.e., epigenetic effects).

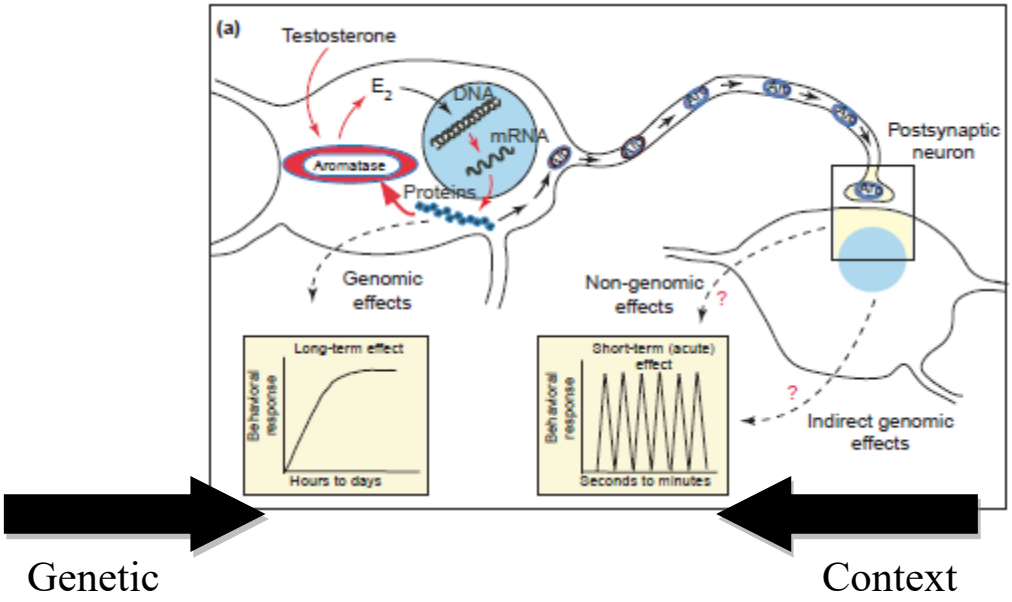

Figure 4. Example of the interaction between genetic and contextual factors that work to determine mating and parenting behaviors (Balthazart and Ball, 2006).

It is likely that gender differences exist with regard to the potential flexibility of an individual in partnering and parenting. As human females experience internal gestation, share a direct hormonal transition with the infant during pregnancy and childbirth, and often experience a prolonged hormonal connection with the infant through breastfeeding, female parenting behavior has been shaped by significant evolutionary pressures to be maintained under a wide range of environmental challenges. On the other hand, male parental behavior would likely have been selected for flexibility as different social and environmental variables would present multiple avenues by which a male could maximize their reproductive value. Studies have supported this suggestion by showing that the behavior of the father, more so than the mother, is affected by contextual features, such as marital satisfaction (Belsky, 1996) and co-parental relationship quality

(Fagan and Palkovitz, 2011). In a similar vein, it has been shown that relationship satisfaction in men increases with increased frequency of pair-bonding behaviors, such as kissing and cuddling (Heiman et al., 2011). As paternal behavior seems to be sensitive to features of partner interaction, it is essential to understand the factors influencing a male's feelings of involvement and security in the family context. This study will include questionnaire data aimed at disentangling the role of the partner from the father.

1.6. Social Implications

The ability to form close social relationships is likely to develop from the foundation of the parent-infant bond. For instance, children that experience more synchronous parental care (responsive caring by both parents in concert) during early infancy were found to be better able to adapt to social situations and negotiate close friendships (Gordon et al., 2010). The OT levels in these children were stable over time and higher levels were associated with securely attached relationships. Efforts to establish and maintain healthy family dynamics should be a priority even before the birth of a child. Results of this research may provide information that can be disseminated by healthcare professionals, childcare providers, and other venues of social and family support to the benefit of the entire family.

1.7. Objectives

Selecting a good parenting partner can directly affect the resulting number of offspring as well as the likelihood of their developmental success. Though maybe not a conscious process, a male may evaluate the resources provided by a female as well as his own investment potential and personal disposition. I will test whether my sample of participants can be assigned to one of three possible male mating strategies - Bachelor,

Provider, and Direct Father. I will also compare the 3-Group model to the alternative 2x2 model of RS-Partner/RS-Parent, where partnering and parenting behaviors will be evaluated separately. Men in the Bachelor group will display characteristics consistent with short-term mating strategies and their associated physiologies, while men in the Direct Father group will display characteristics and physiology consistent with long-term mating strategies. Men in the Provider group will display a mixed strategy with their physiology responding more to the current context than the other groups and maintain T and AVP levels that are similar to men in the Bachelor group.

The biological basis of these strategies will be demonstrated by measurement of hormonal levels and genetic variability through a laboratory based paternal behavior challenge (interaction with a RealCare baby doll) and mate response challenge (view romantic videos with partner). Hormone measurement will include T, CORT, OT, and AVP (Bos et al., 2012). Genetic analysis will include genetic variation of the androgen receptor, as well as the *OXTR*, *CD38*, and *AVPR1A* receptors (Feldman et al., 2012, 2011; Prichard et al., 2007; Walum et al., 2012, 2008).

I expect that baseline hormone levels will vary in men with differing mating strategies; supporting focus on mate access (Bachelor – sexual behavior), exclusive parenting goals (Direct Father – nurturing behavior), or a combination of the two (Provider – sexual and nurturing behavior). When testing each of the three mating strategies in the paternal behavior challenge, I expect that men in the Direct father and Provider groups will experience the greatest changes in hormone levels over the course of the session. These type of changes for men are likely rooted in the findings that T is affected by infant contact (Alvergne et al., 2009; Storey et al., 2011) and OT levels

change when a social interaction requires attention and improvement (van Anders et al., 2011).

1.8. Summary of design

For all experiments, adult males, between the ages of 20-45 years old (with- and without-children), were recruited by community notice in various parenting and medical venues. Participants were considered to be in pair-bonded relationships if the relationship were a minimum of 2 years in length (as suggested in Marazziti and Canale, 2004). All participants resided in St. John's, Newfoundland, Canada at the time of study. Participants filled out a number of questionnaires on parenting, pair-bonding and individual histories and they had blood samples taken before and after the experimental sessions. The two sessions consisted of (a) watching two short romantic movies with their partners and (b) caring for a RealCare baby doll. A final note about the experimental sessions, in order to clarify the primary caregiving role of the participant and prevent sexual signals between partners from interfering with study results, men in this study were alone with the infant/doll during experimental sessions and alone with the partner during the video sessions.

2. Overview of Chapters

2.1. ***Chapter 1*** Male Reproductive Strategies: Relationships between Testosterone, Androgen Receptor Gene, and Male Reproductive Behaviors

Topic

I evaluated individual behavioral differences in men with my proposed comprehensive model of male reproductive strategies (3-Group Model) and compared those results to an alternative model that differentiates between partnering and parenting characteristics (RS-Partnering/RS-Parenting Model) to relate reproductive behavior to individual differences in testosterone levels and number of CAG repeats of the androgen receptor gene.

Hypotheses

Analyses of behavioral data expected to reveal physiological patterns of hormone levels and genotypes consistent with the proposed 3-Group Model and/or the RS-Partner/RS model as determined from K-means cluster analyses. Men in the Bachelor group are expected to display high baseline T and be less responsive to the infant caregiving task (i.e., no significant change in T). Men in the Direct Father group are expected to display low baseline T and be more responsive to the infant caregiving task (i.e., decrease in T). Men in the Provider group are expected to display a mixture of characteristics and respond the most flexibly to both the infant caregiving and romantic interaction tasks.

2.2. ***Chapter 2*** Relationships between Peripheral Levels of Oxytocin and Vasopressin, Oxytocin (*OXTR*), *CD38*, and Vasopressin (*AVPR1A*) Receptor Genes, and Male Reproductive Strategies

Topic

I will use the results of the video and baby doll sessions to evaluate fit of 3-Group Model and the RS-Partner/RS-Parent Model generated in Chapter 1 in relation to OT and AVP levels, as well as their associated hormone receptor genotypes (*OXTR*, *AVPR1A*, and *CD38*).

Hypotheses

The 3-Group and the RS-Partner/RS-Parent models will be assessed in relation to peripheral levels of OT and AVP and variation in hormone receptor genotypes. I expect OT levels to be higher for men carrying genotypes previously associated with nurturant and/or partner-oriented behavior. Similarly, I expect men carrying fewer base pairs for *AVPR1A*-rs1 and/or fewer base pairs (possibly 334 bp specifically) for *AVPR1A*-rs3 to display less responsive partner and parenting behaviors, as well as higher AVP levels.

2.3. ***Chapter 3*** Relationships between male reproductive behaviors and cortisol levels and cortisol interactions with testosterone, oxytocin, vasopressin.

Topic

In order to better understand the relationship(s) between CORT and the often-cited hormones T, OT, and, to a lesser extent, AVP, I examined the changes in hormone levels and behavioral responses of men in the context of a partnering and a parenting challenge. Hormone levels were analyzed as ratios of two hormones to evaluate the

potential effect that CORT has on each hormone.

Methods

Building on data presented in Chapter 1 and 2, T, CORT, OT, and AVP levels were evaluated as ratios in GLM of the proposed 3-Group model and the alternative RS-Partnering/RS-Parenting model.

Hypotheses

Reproductive groups will differ in the direction of the change in each hormone with men in the Bachelor group displaying markers of short-term mating strategies (e.g., greater increase in T/CORT ratio than other men) and those changes will be specific to experimental sessions.

Co-Authorship Statement

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References

Alvergne, A., Faurie, C., Raymond, M., 2009. Father-offspring resemblance predicts paternal investment in humans. Anim. Behav. 78, 61–69. https://doi.org/10.1016/j.anbehav.2009.03.019

Apicella, C.L., Marlowe, F.W., 2007. Men's Reproductive Investment Decisions: Mating, Parenting, and Self-perceived Mate Value. Hum. Nat. 18, 22–34. https://doi.org/10.1007/BF02820844

Apicella, C.L., Marlowe, F.W., 2004. Perceived mate fidelity and paternal resemblance predict men's investment in children. Evol. Hum. Behav. 25, 371–378. https://doi.org/10.1016/j.evolhumbehav.2004.06.003

Atzil, S., Hendler, T., Feldman, R., 2011. Specifying the neurobiological basis of human attachment: Brain, hormones, and behavior in synchronous and intrusive mothers. Neuropsychopharmacology 36, 2603–2615. https://doi.org/10.1038/npp.2011.172

Bakermans-Kranenburg, M.J., van Ijzendoorn, M.H., 2008. Oxytocin receptor (*OXTR*) and serotonin transporter (5-HTT) genes associated with observed parenting. Soc. Cogn. Affect. Neurosci. 3, 128–134. https://doi.org/10.1093/scan/nsn004

Balthazart, J., Ball, G.F., 2006. Is brain estradiol a hormone or a neurotransmitter? Trends Neurosci. 29, 241–249. https://doi.org/10.1016/j.tins.2006.03.004

Bedgood, D., Boggiano, M.M., Turan, B., 2014. Testosterone and social evaluative stress : The moderating role of basal cortisol. Psychoneuroendocrinology 47, 107–115. https://doi.org/10.1016/j.psyneuen.2014.05.007

Belsky, J., 1996. Parent, infant, and social-contextual antecedents of father-son attachment security. Dev. Psychol. 32, 905–913. https://doi.org/10.1037/0012-1649.32.5.905

Bird, B.M., Geniole, S.N., Little, A.C., Moreau, B.J.P., Ortiz, T.L., Goldfarb, B., Bonin, P.L., Carré, J.M., 2017. Does Exogenous Testosterone Modulate Men's Ratings of Facial Dominance or Trustworthiness? Adapt. Hum. Behav. Physiol. 3, 365–385. https://doi.org/10.1007/s40750-017-0079-7

Booth, A., Dabbs, J.M., 1993. Testosterone and men' s mariages. Soc. Forces 72, 463–477.

Bos, P. a, Terburg, D., van Honk, J., 2010. Testosterone decreases trust in socially naive humans. Proc. Natl. Acad. Sci. U. S. A. 107, 9991–5. https://doi.org/10.1073/pnas.0911700107

Bos, P.A., Panksepp, J., Bluthé, R.M., Honk, J. van, 2012. Acute effects of steroid hormones and neuropeptides on human social-emotional behavior: A review of single administration

studies. Front. Neuroendocrinol. 33, 17–35. https://doi.org/10.1016/j.yfrne.2011.01.002

Bribiescas, R.G., 2011. An evolutionary and life history perspective on the role of males on human futures. Futures 43, 729–739. https://doi.org/10.1016/j.futures.2011.05.016

Broude, G.J., 1983. Male-Female Relationships in Cross-Cultural Perspective: A Study of Sex and Intimacy. Cross-Cultural Res. 18, 154–181. https://doi.org/10.1177/106939718301800204

Champagne, F.A., 2008. Epigenetic mechanisms and the transgenerational effects of maternal care. Front. Neuroendocrinol. 29, 386–397. https://doi.org/10.1016/j.yfrne.2008.03.003

Clutton-Brock, T.H., Parker, G.A., 1992. Potential reproductive rates and the operation of sexual selection. Q. Rev. Biol. 67, 437–456. https://doi.org/10.1086/417793

Delville, Y., Mansour, K.M., Ferris, C.F., 1996. Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus. Physiol. Behav. 60, 25–29. https://doi.org/10.1016/0031-9384(95)02246-5

Ditzen, B., Schaer, M., Gabriel, B., Bodenmann, G., Ehlert, U., Heinrichs, M., 2009. Intranasal Oxytocin Increases Positive Communication and Reduces Cortisol Levels During Couple Conflict. Biol. Psychiatry 65, 728–731. https://doi.org/10.1016/j.biopsych.2008.10.011

Ebstein, R.P., Israel, S., Lerer, E., Uzefovsky, F., Shalev, I., Gritsenko, I., Riebold, M., Salomon, S., Yirmiya, N., 2009. Arginine vasopressin and oxytocin modulate human social behavior. Ann. N. Y. Acad. Sci. 1167, 87–102. https://doi.org/10.1111/j.1749-6632.2009.04541.x

Edelstein, R.S., Chopik, W.J., Kean, E.L., 2011. Sociosexuality moderates the association between testosterone and relationship status in men and women. Horm. Behav. 60, 248–255. https://doi.org/10.1016/j.yhbeh.2011.05.007

Fagan, J., Palkovitz, R., 2011. Coparenting and relationship quality effects on father engagement: Variations by residence, romance. J. Marriage Fam. 73, 637–653. https://doi.org/10.1111/j.1741-3737.2011.00834.x

Feldman, R., Gordon, I., Influs, M., Gutbir, T., Ebstein, R.P., 2013. Parental Oxytocin and Early Caregiving Jointly Shape Children ' s Oxytocin Response and Social Reciprocity 38, 1154–1162. https://doi.org/10.1038/npp.2013.22

Feldman, R., Gordon, I., Zagoory-Sharon, O., 2011. Maternal and paternal plasma, salivary, and urinary oxytocin and parent-infant synchrony: Considering stress and affiliation components of human bonding. Dev. Sci. 14, 752–761. https://doi.org/10.1111/j.1467-7687.2010.01021.x

Feldman, R., Zagoory-Sharon, O., Weisman, O., Schneiderman, I., Gordon, I., Maoz, R., Shalev, I., Ebstein, R.P., 2012. Sensitive parenting is associated with plasma oxytocin and polymorphisms in the *OXTR* and CD38 genes. Biol. Psychiatry 72, 175–181. https://doi.org/10.1016/j.biopsych.2011.12.025

Fernandez-Duque, E., Valeggia, C.R., Mendoza, S.P., 2009. The Biology of Paternal Care in Human and Nonhuman Primates. Annu. Rev. Anthropol. 38, 115–130. https://doi.org/10.1146/annurev-anthro-091908-164334

Fouts, H.N., Hewlett, B.S., Lamb, M.E., 2005. Parent-offspring weaning conflicts among the Bofi farmers and foragers of Central Africa. Curr. Anthropol. 46, 29–50. https://doi.org/10.1086/425659

French, J.A., Cavanaugh, J., Mustoe, A.C., Carp, S.B., Womack, S.L., 2018. Social Monogamy in Nonhuman Primates: Phylogeny, Phenotype, and Physiology. J. Sex Res. 55, 410–434. https://doi.org/10.1080/00224499.2017.1339774

Geary, D.C., 2000. Evolution and proximate expression of human paternal investment. Psychol. Bull. 126, 55–77. https://doi.org/10.1037/0033-2909.126.1.55

Gettler, L.T., McKenna, J.J., McDade, T.W., Agustin, S.S., Kuzawa, C.W., 2012. Does Cosleeping Contribute to Lower Testosterone Levels in Fathers? Evidence from the Philippines. PLoS One 7. https://doi.org/10.1371/journal.pone.0041559

Gonzaga, G.C., Turner, R.A., Keltner, D., Campos, B., Altemus, M., 2006. Romantic love and sexual desire in close relationships. Emotion 6, 163–179. https://doi.org/10.1037/1528-3542.6.2.163

Gordon, I., Zagoory-Sharon, O., Leckman, J.F., Feldman, R., 2010. Oxytocin, cortisol, and triadic family interactions. Physiol. Behav. 101, 679–684. https://doi.org/10.1016/j.physbeh.2010.08.008

Gouin, J.P., Carter, C.S., Pournajafi-Nazarloo, H., Glaser, R., Malarkey, W.B., Loving, T.J., Stowell, J., Kiecolt-Glaser, J.K., 2010. Marital behavior, oxytocin, vasopressin, and wound healing. Psychoneuroendocrinology 35, 1082–1090. https://doi.org/10.1016/j.psyneuen.2010.01.009

Gray, P.B., 2003. Marriage, Parenting, and Testosterone Variation Among Kenyan Swahili Men. Am. J. Phys. Anthropol. 122, 279–286. https://doi.org/10.1002/ajpa.10293

Heiman, J.R., Long, J.S., Smith, S.N., Fisher, W.A., Sand, M.S., Rosen, R.C., 2011. Sexual satisfaction and relationship happiness in midlife and older couples in five countries. Arch. Sex. Behav. 40, 741–753. https://doi.org/10.1007/s10508-010-9703-3

Insel, T.R., Young, L.J., 2001. The neurobiology of attachment. Nat. Rev. Neurosci. 2, 129–136. https://doi.org/10.1038/35053579

Kagerbauer, S.M., Martin, J., Schuster, T., Blobner, M., Kochs, E.F., Landgraf, R., 2013. Plasma Oxytocin and Vasopressin do not Predict Neuropeptide Concentrations in Human Cerebrospinal Fluid. J. Neuroendocrinol. 25, 668–673. https://doi.org/10.1111/jne.12038

Kleiman, D.., Malcolm, J.., 1970. The Evolution of Male Parental Investment in Mammals. Parent. Care Mamm. 347–387. https://doi.org/10.1007/978-1-4613-3150-6

Kuzawa, C.W., Gettler, L., Muller, M.N., Mcdade, T.W., Feranil, B., 2009. Fatherhood, Pairbonding and Testosterone in the Philippines. Horm. Behav. 56, 429–435. https://doi.org/10.1016/j.yhbeh.2009.07.010.

Lucassen, N., Tharner, A., Van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Volling, B.L., Verhulst, F.C., Lambregtse-Van den Berg, M.P., Tiemeier, H., 2011. The Association Between Paternal Sensitivity and Infant-Father Attachment Security: A Meta-Analysis of Three Decades of Research. J. Fam. Psychol. 25, 986–992. https://doi.org/10.1037/a0025855

Marazziti, D., Canale, D., 2004. Hormonal changes when falling in love. Psychoneuroendocrinology 29, 931–936. https://doi.org/10.1016/j.psyneuen.2003.08.006

Marlowe, F., 2000. Paternal investment and the human mating system. Behav. Processes 51, 45–61. https://doi.org/10.1016/S0376-6357(00)00118-2

Maurer, T.W., Pleck, J.H., Rane, T.R., 2013. Parental and Reflected-Appraisals : Measurement Identity and Gender Dynamics 63, 309–321.

McCullough, M.E., Churchland, P.S., Mendez, A.J., 2013. Problems with measuring peripheral oxytocin: Can the data on oxytocin and human behavior be trusted? Neurosci. Biobehav. Rev. 37, 1485–1492. https://doi.org/10.1016/j.neubiorev.2013.04.018

Melis, M.R., Argiolas, A., 2011. Central control of penile erection: A re-visitation of the role of oxytocin and its interaction with dopamine and glutamic acid in male rats. Neurosci. Biobehav. Rev. 35, 939–955. https://doi.org/10.1016/j.neubiorev.2010.10.014

Muller, M.N., Marlowe, F.W., Bugumba, R., Ellison, P.T., 2009. Testosterone and paternal care in East African foragers and pastoralists. Proc. Biol. Sci. 276, 347–354. https://doi.org/10.1098/rspb.2008.1028

Nargund, G., 2009. Declining birth rate in Developed Countries: A radical policy re-think is required. Facts, views Vis. ObGyn 1, 191–3.

Neumann, I.D., 2008. Brain oxytocin: A key regulator of emotional and social behaviours in both females and males. J. Neuroendocrinol. 20, 858–865. https://doi.org/10.1111/j.1365-2826.2008.01726.x

Palombit, R.A., 1999. Infanticide and the evolution of pair bonds in nonhuman primates. Evol. Anthropol. 7, 117–129. https://doi.org/10.1002/(SICI)1520-6505(1999)7:4<117::AID-EVAN2>3.0.CO;2-O

Pleck, J.H., 2007. Why could father involvement benefit children? Theoretical perspectives. Appl. Dev. Sci. 11, 196–202. https://doi.org/10.1080/10888690701762068

Ponzi, D., Zilioli, S., Mehta, P.H., Maslov, A., Watson, N. V., 2016. Social network centrality and hormones: The interaction of testosterone and cortisol. Psychoneuroendocrinology 68, 6–13. https://doi.org/10.1016/j.psyneuen.2016.02.014

Prichard, Z.M., Mackinnon, A.J., Jorm, A.F., Easteal, S., 2007. AVPR1A and *OXTR* polymorphisms are associated with sexual and reproductive behavioral phenotypes in humans. Mutation in brief no. 981. Online. Hum. Mutat. 28, 1150. https://doi.org/10.1002/humu.9510

Quinlan, R.J., Quinlan, M.B., 2008. Human lactation, pair-bonds, and alloparents : AA cross-cultural analysis. Hum. Nat. 19, 87–102. https://doi.org/10.1007/s12110-007-9026-9

Quinlan, R.J., Quinlan, M.B., 2007. Evolutionary ecology of human pair-bonds: Cross-cultural tests of alternative hypotheses. Cross-Cultural Res. 41, 149–169. https://doi.org/10.1177/1069397106298893

Reimers, L., Diekhof, E.K., 2015. Testosterone is associated with cooperation during intergroup competition by enhancing parochial altruism. Front. Neurosci. 9, 1–9. https://doi.org/10.3389/fnins.2015.00183

Ross, C., MacLarnon, A., 2000. The evolution of non-maternal care in anthropoid primates: A test of the hypotheses. Folia Primatol. 71, 93–113. https://doi.org/10.1159/000021733

Schacht, R., Kramer, K.L., 2019. Are We Monogamous? A Review of the Evolution of Pair-Bonding in Humans and Its Contemporary Variation Cross-Culturally. Front. Ecol. Evol. 7, 1–10. https://doi.org/10.3389/fevo.2019.00230

Scheele, D., Striepens, N., Gunturkun, O., Deutschlander, S., Maier, W., Kendrick, K.M., Hurlemann, R., 2012. Oxytocin Modulates Social Distance between Males and Females. J. Neurosci. 32, 16074–16079. https://doi.org/10.1523/JNEUROSCI.2755-12.2012

Sroufe, L.A., 2005. Attachment and development: A prospective, longitudinal study from birth to adulthood. Attach. Hum. Dev. 7, 349–367. https://doi.org/10.1080/14616730500365928

Storey, A.E., Alloway, H., Walsh, C.J., 2020. Dads: Progress in understanding the neuroendocrine basis of human fathering behavior. Horm. Behav. 119, 104660. https://doi.org/10.1016/j.yhbeh.2019.104660

Storey, A.E., Noseworthy, D.E., Delahunty, K.M., Halfyard, S.J., Mckay, D.W., 2011. The effects of social context on the hormonal and behavioral responsiveness of human fathers. Horm. Behav. 60, 353–361. https://doi.org/10.1016/j.yhbeh.2011.07.001

Storey, A.E., Walsh, C.J., Quiton, R.L., Wynne-Edwards, K.E., 2000. Hormonal correlates of paternal responsiveness in new and expectant fathers. Evol. Hum. Behav. 21, 79–95. https://doi.org/10.1016/S1090-5138(99)00042-2

Strathearn, L., Fonagy, P., Amico, J., Montague, R., 2011. To Infant Cues. Read 34, 2655–2666. https://doi.org/10.1038/npp.2009.103.Adult

Taylor, S.E., Saphire-Bernstein, S., Seeman, T.E., 2010. Are Plasma Oxytocin in Women and Plasma Vasopressin in Men Biomarkers of Distressed Pair-Bond Relationships? Psychol. Sci. 21, 3–7. https://doi.org/10.1177/0956797609356507

Trivers, R., 1972. Parental Investment and Sexual Selection, in: Bernard Campbell, E.A. (Ed.), Sexual Selection and the Descent of Man, 1871-1971. Aldine Publishing Company, Chicago, IL, pp. 136–179.

van Anders, S.M., Tolman, R.M., Jainagaraj, G., 2014. Examining How Infant Interactions Influence Men's Hormones, Affect, and Aggression Using the Michigan Infant Nurturance Simulation Paradigm. Fathering 12, 143–160. https://doi.org/10.3149/fth.1202.143

van Anders, S.M., Edelstein, R.S., Wade, R.M., Samples-Steele, C.R., 2012a. Descriptive experiences and sexual vs. Nurturant aspects of cuddling between adult romantic partners. Arch. Sex. Behav. 42, 553–560. https://doi.org/10.1007/s10508-012-0014-8

van Anders, S.M., Goldey, K.L., 2010. Testosterone and partnering are linked via relationship status for women and "relationship orientation" for men. Horm. Behav. 58, 820–826. https://doi.org/10.1016/j.yhbeh.2010.08.005

van Anders, S.M., Goldey, K.L., Kuo, P.X., 2011. The Steroid/Peptide Theory of Social Bonds: Integrating testosterone and peptide responses for classifying social behavioral contexts. Psychoneuroendocrinology 36, 1265–1275. https://doi.org/10.1016/j.psyneuen.2011.06.001

van Anders, Sari M., Hamilton, L.D., Schmidt, N., Watson, N. V., 2007. Associations between testosterone secretion and sexual activity in women. Horm. Behav. 51, 477–482. https://doi.org/10.1016/j.yhbeh.2007.01.003

van Anders, Sari M, Hamilton, L.D., Watson, N. V., 2007. Multiple partners are associated with higher testosterone in North American men and women. Horm. Behav. 51, 454–459. https://doi.org/10.1016/j.yhbeh.2007.01.002

van Anders, S.M., Tolman, R.M., Volling, B.L., 2012b. Baby cries and nurturance affect testosterone in men. Horm. Behav. 61, 31–36. https://doi.org/10.1016/j.yhbeh.2011.09.012

Walum, H., Lichtenstein, P., Neiderhiser, J.M., Reiss, D., Ganiban, J.M., Spotts, E.L., Pedersen, N.L., Anckarster, H., Larsson, H., Westberg, L., 2012. Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. Biol. Psychiatry 71, 419–426. https://doi.org/10.1016/j.biopsych.2011.09.002

Walum, H., Westberg, L., Henningsson, S., Neiderhiser, J.M., Reiss, D., Igl, W., Ganiban, J.M., Spotts, E.L., Pedersen, N.L., Eriksson, E., Lichtenstein, P., 2008. Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. Proc. Natl. Acad. Sci. U. S. A. 105, 14153–14156. https://doi.org/10.1073/pnas.0803081105

Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The "challenge hypothesis'': theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies." Am. Nat. 136, 829–846. https://doi.org/10.1086/285134

Winking, J., 2010. Biodemography and Social Biology Are men really that bad as fathers ? The role of men ' s investments Are Men Really that Bad as. October 37–41.

Zilioli, S., Ponzi, D., Henry, A., Maestripieri, D., 2015. Testosterone, Cortisol and Empathy: Evidence for the Dual-Hormone Hypothesis. Adapt. Hum. Behav. Physiol. 1, 421–433. https://doi.org/10.1007/s40750-014-0017-x

Chapter 1

Male Reproductive Strategies: Relationships between Testosterone, Androgen Receptor Genes, and Male Reproductive Behaviors

by

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Abstract

Previous literature has shown that testosterone levels vary between men in different partnership and parenting contexts, and these differences can be conceptualized as state (context) vs. trait (individual) characteristics. To evaluate trait differences between individuals, I propose a comprehensive model of male reproductive strategies (3-Group model) and compare it l to an alternative model (RS-Partner/RS-Parent model). I used K-means clustering analyses to assign men to one of three categories based on measures of both partnering and parenting variables: Bachelor, Provider, and Direct Father (3-Group Model). Similarly, the RS-Partnering/RS-Parenting Model separates partnering and parenting variables to create four distinct, but interrelated, categories of individuals. Fifty-two men completed at least one experimental session including questionnaires related to relationships and parenting, blood samples before and after one of two experimental interaction(s) - 1) caring for a robotic baby doll, 2) watching two romantic video clips with their romantic partner (if applicable). Blood samples were assayed for testosterone (T) levels before and after experimental interaction(s). Additionally, blood samples were sequenced for CAG repeat polymorphisms of the androgen receptor gene (*AR*). Men with lower than average number of CAG repeats reported experiencing more control from their partners than men with a higher number of repeats, and those men with a specific low number of repeats (10, 19, 21 & 23 repeats) had higher T levels than other men. K-mean clusters successfully distinguished among three groups: men in the Bachelor group reported demographic data consistent with short-term mating strategies and they had higher baseline T levels than men in the other two groups. Men in the Provider group exhibited a mix of short-term and long-term mating strategies whereas men in the Direct Father group generally exhibited long-term mating strategies. Results indicate that my original 3-Group Model was a better representation of partnering and parenting behaviors and related physiologies for testosterone than the RS-Partnering/RS-Parenting Model, within the context of this population and experimental method.

Keywords: Testosterone, Androgen Receptors, *AR*-CAG Repeats, Reproductive Strategies, Paternal Behavior.

Highlights:

- First study to combine reproductive behavior, endocrinology, and genetics within the context of human reproductive strategies
- Identified two possible models of reproductive strategies
- Suggest that partnership may be the gateway for the development of paternal physiology and behavior
- Support the use of the RealCare Baby Doll as an infant proxy, allowing for more accurate study of a broad demographic

1. Introduction

Male reproductive behavior is often described as a suite of characteristics displayed by a homogenous population of males with a narrow range of static behaviors and associated physiological characteristics. Examples of such limited perspective include the notion that all males will achieve reproductive success by utilizing the same sexual strategies (e.g., mate with as many females as possible without providing parental care to the offspring). However, competition among males can result in some males achieving high levels of reproductive success (those endowed with a competitive advantage) while others do not. Since optimal reproductive success requires prioritization of resources (Ellison, 2003; Kaplan and Gangestad, 2004), energy can be allotted differentially to strategies such as mate access/retention and/or parental care of offspring as alternative forms of competition for mates. Selection of reproductive priorities requires consideration of many trade-offs inherent in reproduction, including individual quality and the quality of the potential mate, the time investment required for each potential mate, the relative

presence of similar competitors, and the availability of resources necessary to engage in parental care and/or resource acquisition (Gangestad and Simpson, 2000).

Biologists have long recognized distinct male reproductive strategies in various animal species that allow maximization of fitness, including significant intraspecific differences in behavioral and physiological characteristics. For example, there are two reproductive strategies present in male Atlantic Salmon (*Salmo salar)*: the anadromous male and the mature parr (Fleming, 1996). The anadromous males are fully grown, ~75 cm in length, display secondary sexual characteristics (higher testosterone,T), and return from sea to compete for mating females. Mature parr, ~15 cm in length and without secondary sexual characteristics (lower T), are first-year males that remain in the natal river and parasitize the breeding efforts of the anadromous males by sneaking in to fertilize eggs. Other examples of alternative reproductive strategies include behavioral differences between home-territory vs. polyterriorial males in Pied flycatchers (*Ficedula hypoleuca,* Silverin & Wingfield, 1982), as well as behavioral and physiological differences between resident and transient male degus (*Octodon degus;* Soto-Gamboa et al., 2005)*,* and between hierarchically status-ranked male chimpanzees (*Pan troglodytes schweinfurthii,* Muller & Wrangham, 2004).

Researchers have similarly examined differences in human male reproductive strategies and their corresponding behavioral and physiological differences. Discussions of the theoretical origin of the transition from male promiscuity to pair-bonding have included the concept that less physically competitive males may have utilized an alternative reproductive strategy (e.g., paternal care) so that they could compete with dominant males for mating opportunities (see Gavrilets, 2012). Alternative reproductive strategies can incur costs including physiological effects such as decreased immune function and increased risk of illness associated with high T (Booth et al., 2005). Due to the cost of reproductive strategies, men can invest in either long-term partnering and parenting (‘dads’) or short-term mating opportunities (‘cads’) as resources and time may limit

engagement in both strategies simultaneously (Cashdan, 1993). James et al. (2012) found that characteristics such as early sexual debut, sexual risk taking, and self-perceived mate value were influenced by interactions between gender and natal family stress. Specifically, results indicated gender differences for earlier pubertal maturation in girls with absentee fathers and earlier sexual debut for boys with higher self-perceived mate value. Additionally, father engagement (i.e., quality of father-mother co-parenting, and by extension father-infant interaction) was shown to be more predictive of healthy child development than father residential status (Fagan and Palkovitz, 2011). Taken together, these results suggest that reproductive physiology (i.e., genetic disposition and endocrine function) work in unison with socio-environmental cues to fine-tune developing reproductive strategies in humans.

1.1 Reproductive behavior studies

One way to simplify the continuum of individual male reproductive behavior is to categorize men by behavioral characteristics. In one example, researchers considered four categories: the "new, involved father"; the "good provider"; the "deadbeat dad"; and the "paternity-free man" (Marks & Palkovitz, 2004). The "new, involved father" was defined as an equal partner to the mother, including significant involvement in household and childcare responsibilities. The "good provider" was defined as a financial and personal support to the mother's efforts of household management and parenting, sometimes described as physically present, but psychologically absent (Fagan and Palkovitz, 2011). The "deadbeat dad" was defined as a father who is not meeting the personal or financial responsibilities of fatherhood or partnership. The "paternity-free man" was a man who has avoided familial responsibilities entirely. For the purposes of my study, I chose to narrow the proposed categories of men to the "new, involved father"; the "good provider", and the "paternity-free man". I decided not to focus on the "deadbeat dad" as they are difficult to recruit as participants, and there is a high probability of misrepresentation of their contributions to parenting during self-report.

Similar to partitioning men by parental behaviors, men can also be categorized by their partnership-related behaviors. Researchers proposed three distinct emotion and motivation processes in the brain related to pair-bonds and parenting: lust, attraction and attachment (Fisher et al., 2006). They suggest that these three brain processes can operate simultaneously or sequentially and that they have evolved to support individuals as they maximize their reproductive success by allowing an individual to respond to multiple contextual stimuli at once. Similarly, a study evaluating gender differences between short-term and long-term mating strategies found that males (and females) select partners based on their suitability for the currently selected relationship type (Stewart et al., 2000). In contrast to the idea of a male selecting a single current strategy, researchers have argued that limiting evaluation of male reproductive behavior to either short-term or long-term strategies creates a false dichotomy (Jackson and Kirkpatrick, 2007). Their study examined behavioral characteristics, as reported in an expanded Sociosexual Orientation Inventory with additional data pertaining to reproductive strategies. Results suggest that men may be pursuing short-term and long-term strategies simultaneously, as resources and time allow. Taken together, these studies suggest that individuals consider multiple factors when weighing the potential benefits of a prospective mate relative to their current context and future reproductive goals, and may flexibly alternate between available strategies.

1.2. Reproductive behavior and testosterone

Recent studies of human male reproduction have examined differences in behaviors and physiological markers, such as the hormone testosterone (T), in the context of relationships and parenting. Studies comparing single males to men in pair-bonded relationships have found a general trend for pair-bonded males to have lower T than their single counterparts (van Anders, et al., 2007; Booth & Dabbs, 1993; Burnham et al., 2003; Marazziti & Canale, 2004; Mazur & Michalek, 1998; van Anders et al., 2011). However, the trend for lower T in pair-bonded men is complicated by individual trait and state differences. Trait characteristics refer to features inherent

to the individual, while state characteristics refer to transient features of the individual relative to their current social context.

One study of T and partnership traits found that T levels were higher in men who preferred short-term relationships than in men who preferred long-term relationships (casual/single vs. long-term relationship; van Anders & Goldey, 2010). In essence, their results suggest that men's hormone levels vary less in response to the characteristics of their relationship (i.e., sexual frequency, perceived partner quality, etc.) than to their own preferred relationship context. Other examples of trait differences include interest in additional sexual partners (van Anders et al., 2010; McIntyre et al., 2006), levels of sexual desire (Edelstein et al., 2011), and dominance and impulsivity which are positively correlated with T levels (Carré and Olmstead, 2015). Likewise, a laboratory study of competitive interactions found that status seeking tendencies are positively correlated with T levels when individuals are in their preferred status position (i.e., high status seeking men in high status positions and low status seeking men in low status positions; Josephs, Sellers, & Newman, 2006). Examples of state differences in the context of male T levels in pair-bonded relationships include lower T levels in pair-bonded relationships (McIntyre et al., 2006), a negative relationship between T and relationship quality (Edelstein et al., 2014), decreased T levels with satiation of desire for new sexual partners (Puts et al., 2015), increased T at the end of a pair-bond (Mazur and Michalek, 1998), and sexual orientation and types of intimacy (T increases in sexual intimacy vs. T decreases in nurturant intimacy) present in the pair-bond (van Anders et al., 2012a).

In general, fathers have lower T than non-fathers (Alvergne et al., 2009; Gettler et al., 2011; Perini et al., 2012; Storey et al., 2000) and fathers with lower T levels show more empathetic responses toward infants (Fleming et al., 2002). Further, T levels decrease following successful paternal care but remain unchanged following unsuccessful paternal care (van Anders et al., 2012b). Hormone differences also appear between geographically and culturally dissimilar

groups of fathers. Monogamously married fathers had lower T than polygynously married fathers (Gray, 2003), fathers in societies with more contact with their children had lower T than fathers without such contact (Muller et al., 2009), and fathers that participated in significant amounts of direct childcare had lower T than fathers that did not spend significant time caring for their children (Gettler et al., 2011). Additionally, a study of men as they transitioned from single males to married fathers demonstrated that the reduced T of married fathers does not simply reflect individual differences or is an artifact of age, but rather is an effect of the relationship (Gettler et al., 2012). However, complicated results also exist within this behavioral research. For example, father T level decreases may be confounded by individual investment in continued mate access (Mascaro et al., 2013), participation in polygynous marriage (Gray, 2003), or a lack of involvement/access during the course of the pregnancy (Saxbe et al., 2017). Additionally, high sexual frequency during the transition to fatherhood has been found to moderate the typical T decline of fatherhood (Gettler et al., 2013). Taken together, the current understanding of the relationships between pair-bonding and parental experience encourages further exploration of the potential sources of conflicting hormonal and behavioral representations of male reproductive strategies.

One theory that addresses the source of potential conflict in hormonal response to relationships and parenting is the Steroid/Peptide Theory of Social Bonds (van Anders et al., 2011). The key aspect of this theory is the contextual effect of the social interaction on the hormonal response. For example, daily T levels are lower in fathers when they spent more time with their infants/toddlers compared to other fathers who spent less time with them (Gettler et al., 2012; Gettler et al., 2011; for cultural context see Gray, 2003; Muller et al., 2009). Testosterone also decreases more during the transition to fatherhood, when men report higher relationship quality with the mother than in new fathers that do not express high partner quality (Perini et al., 2012).

Although T levels decline during transitions to partnering and parenting, changes in T differ greatly in other behavioral contexts. For example, T levels increase following physical competition (Carré and Olmstead, 2015; Trumble et al., 2012). Furthermore, men who participate in multiple-partner relationships have higher testosterone than both uni-partnered men and un-partnered men (van Anders et al., 2007; McIntyre et al., 2006). However, a recent study of T, sociosexuality, and number of sexual partners found a more complicated relationship between number of partners and T levels. Puts et al. (2015) found that when sexual attitudes and relationship views were controlled for statistically, men reporting more sexual partners had lower T levels than men reporting fewer sexual partners. The authors suggested that T drives the desire for new sexual partners until the desire for sexual novelty has been satisfied. As mentioned previously, frequency of sexual interactions between the parents has been found to moderate T declines in new fathers, such that more sexual interactions are associated with less of a T decline when men become parents (Gettler et al., 2013). These studies help explain some of the conflicting findings; however, they fail to address all of the individual differences that likely exist among men.

To address more of the individual differences in men's hormonal responses, researchers have begun to focus on state and trait characteristics separately as well as their interaction(s). For example, exogenous T increased aggressive behavior but only in dominant and impulsive males (Carré et al., 2017). Another study found a relationship between T and cortisol, and status among executive males (Sherman et al., 2015). Specifically, men with high T and low cortisol were more likely to achieve higher professional status than other combinations of hormone levels. These results suggest that high levels of cortisol mask or moderate the effects of T. Another study of individual differences in hormone levels found that T levels in fathers are dependent on the education level of the father (Jasienska & Ellison, 2012). In this study, men with a college degree had decreasing T levels with increasing number of children, whereas men with less than a college

education had higher levels of T than less educated men, regardless of number of children. Taken together, these studies suggest a complex relationship between individual traits and their associated capacities, interactions with the local social environment, and relative opportunity for social interactions that ultimately determines a male's reproductive physiology.

1.3. Androgen receptor genetics

Developments in genetic sequencing and analyses have encouraged the study of genotypes that may be associated with reproductive behaviors and physiology. Evidence of polymorphisms in the androgen receptor (*AR*) gene include the finding that eight to thirty-seven exon 1 CAG (polyglutamine - nucleotide sequence) repeats are found in healthy men and the number of repeats is inversely associated with *AR* transcriptional activity, which is linked to a range of androgenic somatic and behavioral traits (Campbell et al., 2007; Ryan et al., 2017). Characteristics like body composition are likely affected by the number of CAG repeats by modifying the efficacy of the *AR* receptor (Beilin et al., 2000) and results vary in terms of how the number of repeats affects T levels. A study of *AR* gene polymorphisms, aggression, and reproductive success in African men indicated that men with fewer CAG repeats (CAGn) were more aggressive and had fewer children (Butovskaya et al., 2015). Another study examined changes in T during an interaction with a prospective mate and found that men with fewer CAG repeats and low cortisol levels experienced greater increases in T following a brief conversation with an attractive woman than men with more repeats (Roney et al., 2010). Gettler et al. (2017) found that men who had high androgenicity (elevated T and fewer CAGn) showed elevated likelihood of relationship instability over the 4.5-year study period and were also more likely be relatively uninvolved with childcare as fathers. Taken together, these results suggest that T levels and *AR* gene polymorphisms may be honest indicators of male reproductive strategies, male reproductive success, and possibly even of resultant offspring quality and fitness (see Manning et al., 2003), and therefore may be a target for sexual selection. It is also important to note the

potential for inter-population variation in average number of CAG repeats. Sartor et al. (1999) found that black men were twice as likely as non-Hispanic white men to have fewer than 20 CAG repeats, which was associated with increased body mass.

1.4. Objectives

This study aims to further support the concept of behaviorally and physiologically distinct reproductive strategies in human males by examining differences in individual reproductive behavior, T levels, variation in androgen receptor genes, and their link(s) to social context and life stage. To test my hypotheses, I exposed men to parenting and partnering interactions, collected behavioral data, and intravenous blood samples to examine individual differences in the behavioral, hormonal, and genetic variation in responses of men to relationship and parenting contexts.

Based on previous studies of variation in male reproductive behavior and physiology, I hypothesized that human males have the capacity to express one of multiple reproductive strategies, in response to their current context, and within their individual capacity. I will (a) use K-means clustering to establish and compare either three groups that combine parental and mating strategies (Bachelor, Provider, Direct Father in 3-Group model) or four groups that consider mating and parental strategies separately (high and low partner and parent, RS-Partner/RS-Parent model); (b) determine what behavioral and demographic factors distinguish the groups most clearly (c) with regard to T levels, determine which model best accounts for differences and (d) whether T levels vary with parental status and number of CAG repeats. Specific predictions can be found in Tables 1 and 2.

Table 1. Hypothesized outcomes for 3-Group Model of reproductive strategies

	Reproductive Strategies		
Measures	**Bachelor**	**Provider**	**Direct Father**
Have Kids	No	Yes	Yes
Time with Kids	Low	Moderate	High
Number Partners	High	Moderate	Low
Future Partners	High	Low	Low
Relationship Quality	N/A	High	Moderate
Relationship Care	Low	High	Moderate
Relationship Control	Low	High	Moderate
Baby Doll Score	Low	Moderate	High
Cry Mins	High	Moderate	Low
Testosterone (baseline)	High	Moderate	Low
Testosterone (post-doll)	High	Low	Low
Testosterone (post-video)	High	High	Low

Table 2. Hypothesized demographic data outcomes for RS-Partner

Variables	**High Partner**	**Low partner**
Number of partners	Low	High
Estimated number of future partners	Low	High
Rel. Quality (DAS Total)	High	Low
Relationship care (IBM)	High	Low
Relationship control (IBM)	Low	High

Table 3. Hypothesized demographic data outcomes for RS-Parent

Variables	**High Nurture**	**Low Nurture**
Have kids	More Fathers	Fewer Fathers
Time with kids	High	Low
Baby Doll Score	High	Low
Cry Mins	Low	High

2. Methods

2.1. Study population

Men were recruited from prenatal, breastfeeding, and infant care classes at the Women's Health Centre located in the Health Sciences Centre, St. John's, Newfoundland and Labrador. Participants were also recruited through informational talks and interdepartmental newsletters at Memorial University of Newfoundland, as well as through community flyers, posters, and blood donor clinics. A total of 52 men successfully completed experimental sessions and gave blood samples that provided assayed hormones and genetic material. There were a total 44 men in the partner video session and 46 men in the baby doll session, with 38 men participating in both the video and the doll session. All of the men were English speaking and between the ages of 19 and

45 years old (M = 31.24 $\pm$ 7.31). Participant ages were selected to avoid the hormonal peaks following the end of puberty and the increased hormonal variability in aging males (Kelsey et al., 2014). Participants self-reported their place origin as Newfoundland (n=33), Canada (n=11), and Other (n=8), and their parental status as having a baby (n=11), older children (n=12), or no children (n=29). Interestingly, of the men that reported not having children, 17 reported wanting children in the future, while 12 men reported not wanting children.

2.2. Procedure

Experimental sessions occurred in the Psychology Department of Memorial University from December 2013 to December 2015 between 1200-1600 hours, with subsequent sessions occurring approximately one week later. Men participated in 1-2 experimental sessions and were asked to refrain from caffeine, alcohol, and sexual interaction for the 24-hours prior to their session, and confirmed upon arrival at the session. Participants were also asked to refrain from intense exercise just prior to the session. For all sessions, participants filled out questionnaires related to personal demographics, relationship dynamics, interactions with children, and their experiences during the experimental sessions. Once the questionnaires were complete, I took a venous blood sample for the analyses of T. Blood samples were also sequenced for Androgen Receptor repeat polymorphisms (number of CAG repeats). Following blood samples, there were two experimental interaction sessions: the baby doll interaction and the romantic videos. The baby doll session utilized the RealCare baby doll (see van Anders et al., 2012) as an infant proxy. The video session included the man and his partner (if applicable) watching two romantic video clips (see Steiner, 2011). Approximately 30-mins after the first blood sample, participants gave a second blood sample and reported their experiences during the session.

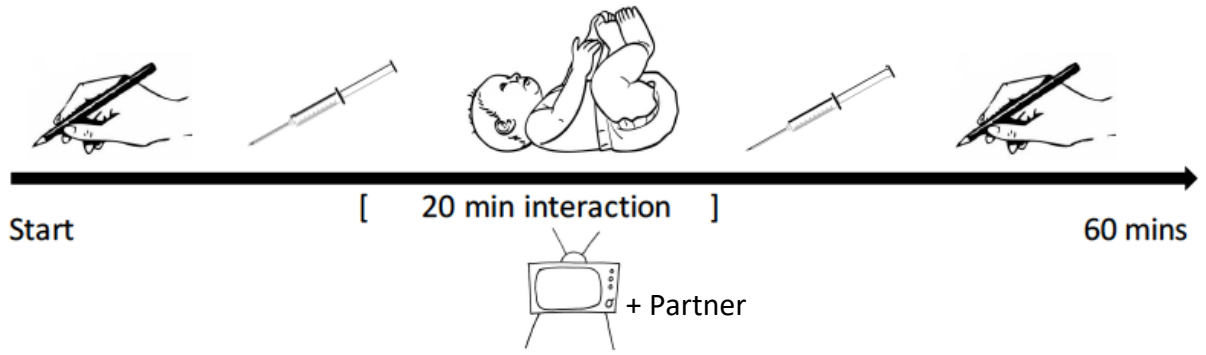


Figure 1. Experimental method for RealCare baby doll and romantic video session.

* note that second blood sample occurred approximately 30-minutes after the first blood sample

2.3. Questionnaire data

Questionnaires included a customized demographic questionnaire, the Dyadic Adjustment Scale (DAS - Spanier, 1976), and the Intimate Bond Measure (IBS –Wilhelm, 1988; see Appendices). The demographic information questionnaire covered topics such as age, weight, smoking habits, profession, place of origin, relationship status, parenting experience, sexual relations and history. Variables were interpreted from the raw demographic data. For example, "Time with Kids" was defined as significant interaction (primary responsible care-giver) with children 1= more than 10h/week, 2= less than 10h/week (Gauthier et al., 2011).

The DAS included thirty-two scaled questions (i.e., 0 =Always Disagree, 5= Always Agree; "Which of the following statements best describes…"; "yes" or "no" questions; etc.) related to interaction quality across relationship topics. Scoring for the DAS is broken down into four categories: perception, affection, consensus, and cohesion; and the total score (described in detail in Spanier, 1976). Although the scoring method for the DAS includes five interdependent subcategories, we decided to use only the total DAS score as a representation of relationship quality for our statistical analyses (as per Walum et al., 2008).

The IBS included 24 questions regarding the participants interaction quality with their partner. Twelve of the questions relate to care behaviors and twelve of the questions relate to control behaviors. For example, participants responded to statements such as “my partner is considerate of me”, “my partner wants to know where I am at all times”. Answers were rated as “true”, “moderately”, “somewhat”, or “not at all”, with higher numbers representing higher levels of care and/or control in the relationship.

2.4. Video session: Pair-bonding interaction

During the video session each man watched two romantic videos (one mildly sexually romantic and one emotionally romantic) with his partner. Videos were selected based on the romantic quality of the interaction between the heterosexual couple portrayed in the video as well as the equality of the interest in the opposite individual. Care was taken to avoid overtly sexual content as it may have interfered with the romatic nature that the videos were intended to capture. The mildly sexually romantic video was an excerpt from *The Notebook* (2004), depicting two estranged lovers reuniting in a mildly sexual scene. The emotionally romantic video was a short film titled *Signs* (2008), depicting an office romance at a distance with the use of “signs” (sheets of paper with short notes on them). There was no nudity or graphic content in either video. Presentation order was counterbalanced between participants. All sessions were approximately 60-minutes long, with 22-minutes of video time and approximately 30-minutes between blood samples.

2.5. RealCare baby doll session: Parenting interaction

The RealCare baby doll approximated a 6-month old male infant (manufactured by RealityWorks; as used in van Anders et al., 2012b, van Anders et al., 2014). It was dressed in a blue shirt and footed pants. Once activated, the doll behaved as directed by its selected care program for each participant, such that every individual experienced the same pattern of behaviors. During the 20-minute interaction the doll ‘requested’ four care events: bottle feed,

burp, diaper change, and gentle rocking. Each man was responsible for identifying and providing the care required. The doll recorded successful vs. unsuccessful care attempts (Doll Score - percentage out of 100), number of minutes spent crying (Cry Mins), and any instances of rough handling (Doll Score - deducted points from total). Data from the doll were downloaded upon completion of the interaction and participants were asked to fill-out a follow-up questionnaire about their experiences during the session (Appendices). The doll has not been formally validated but the change in OT in a small sample of new fathers tested with their own babies in this study was marginally correlated with their OT change with the baby doll ($r = 0.84$, $p = .08$, $n = 5$).

2.6. Sample collection and processing protocol

Blood samples were collected by standard protocol for venous blood collection into 8ml EDTA filled plastic vacutainers. Samples were kept on ice for up to one hour and then centrifuged at 1,6000g for 15-mins. Once centrifuged, plasma was aliquoted into a 2-ml O-ring tubes and stored at -20°C for 1-3 months for enzyme immunoassay of T. The remaining sample (white and red blood cells) was stored at 4°C for up to one week for DNA extraction in batches by salting out method (Miller et al., 1988). Extracted DNA samples were frozen at -20°C for genotyping.

2.7. Testosterone assay

T immunoassays (nmol/mL) were performed at the clinical laboratory at the Health Sciences Hospital at Memorial University, St. John's, Newfoundland. Plasma samples were run in duplicate on an Abbott Diagnostics Architect; 2000SR immunoassay analyzer with a 2nd Generation Testosterone assay kit, following standard protocol as described in the kit insert. The within and between run imprecision for serum testosterone using this method is generally less than 5 percent for samples between 0.5 and 46.0 nmol/L. Run impression is validated regularly for all clinical assays, including these samples.

2.8. Gene sequencing

Gene sequencing was performed at the GAP laboratory of Memorial University, St. John's, Newfoundland. The *AR* gene PCR was performed with the primers forward, 5′-NED-GTGCGCGAAGTGATCCAGAA-3′; and reverse, 5′-TAGCCTGTGGGGCCTCTACG-3′. The master PCR mixes were prepared in a 1.5 ml microcentrifuge tube, and individual reactions were pipetted into 8-well PCR strip tubes. Tubes were capped, vortexed and centrifuged briefly. For each PCR experiment, a negative control (no DNA present) was also included. Tubes were placed in a Veriti thermal cycler* (Applied Biosystems). Prior to amplification of participant DNA, a thermal cycler protocol was verified using control DNA. Polymerase chain reactions were verified using electrophoresis. Agarose gels were prepared using 50 ml of 1x TAE buffer and 1 gram of UltraPure agarose. To prepare for electrophoresis, 3 µl of each PCR product was mixed with 3.5 µl of 5x loading dye and loaded next to 1 µl of 100 base pair DNA ladder (mixed with 3.5 µl loading dye) as a frame of reference. Each gel was run for approximately 25-minutes at 120 V. Gels were viewed under UV light using an AlphaImager EP light cabinet. Excess dNTPs were removed using a mixture of Exonuclease I and Shrimp Alkaline Phosphatase. For each PCR product, 4 µl of product was added to a mixture of 0.5 µl of exonuclease, 0.5 µl of shrimp alkaline phosphatase and 7.5 µl H_2O. The master mix of ExoSAP was prepared in a 1.5 µl microcentrifuge tube over ice. Individual reactions were pipetted into wells of 8-well strip tubes, and capped. Tubes were vortexed, centrifuged briefly and then placed in a Veriti thermal cycler on the ExoSAP protocol.

Two of the above reaction mixtures were created for each ExoSAP product, one using a forward primer and one using a reverse primer. The reactions were added to a 96-well plate. Plates were capped, vortexed, and centrifuged for 30 seconds at 400 rpm. Plates were placed in the Veriti thermal cycler on the ABISeq protocol. After removing the plate from the thermal cycler, ethanol precipitation and programming of the sequencer was completed. Following

sequencing, results were analyzed using Sequencher 5.0 desktop software to align sequences to the human reference sequence for *AR*-1. Chromatographs and base calls could then be visualized and CAGn totaled.

2.9. Statistical analyses- Behavioral Data

All statistical analyses were performed in SPSS version 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Results were considered significant at $p<0.05$. Data exploration was carried out following the protocol described in (Zuur et al., 2010). Behavioral data for all participants were then examined for patterns in questionnaire data by K-means clustering analyses. K-means clustering partitions data into selected number of groups in which each data point belongs to the cluster with the nearest mean. This method of cluster analyses allows for comparison of groups based on hypothesized differences. I began our analyses by selecting three clusters for k-means cluster analysis based on my original hypotheses (3-Group Model). I expected to find significant differences in behaviors that matched the descriptions of my three hypothesized groups: Bachelor, Provider, and Direct Father (per Bonferroni corrections).

In order to evaluate the significance of the clustering results, I also compared my initial hypothesis of three reproductive strategies with the alternative hypothesis that parental and pair-bonding behaviors are not mutually exclusive, meaning that an individual could be high on one or both categories, or neither (RS-Partner/RS-Parent: High vs. Low). For these analyses, I ran K-means clustering on pair-bonding and parental behaviors separately, compared the resulting cluster groups, and performed post-hoc tests for group differences. One-way ANOVAs were used to test differences between the 3-Groups, with Bonferroni correction for multiple tests. T-tests were used for the two group analyses: for RS-Partner and RS-Parent comparisons. In the case of nominal data, Fisher's test was utilized.

2.10. Testosterone levels and AR gene data analyses

To evaluate individual differences in T levels between men, I performed t-tests and ANOVAs of demographic data and T levels. Analyses of the independent variables of behavioral strategies (the 3-Group model and RS-Partner/RS-Parent model) and number of CAG repeats were performed by multivariate GLM of the video and baby doll sessions. T data were analyzed as baseline T (T level prior to experimental interaction), reactive T (T level following experimental interaction), and change T (T level after testing minus T level before interaction, so a positive change indicated an increase). Note that results for percent change in T yielded similar results to absolute change T and was therefore omitted from further analyses. CAG genotype data was calculated as the number of CAG repeats split by mean/median (same value), and as a continuous independent variable.

2.11. Ethical statement

Institutional approval from HREA (Memorial University of Newfoundland) was obtained prior to data collection for this study on August 22, 2013. All subjects gave their informed, verbal and written consent prior to participation. Participants relationship partners gave verbal consent prior to viewing the videos. At the conclusion of each session, participant identification was removed from the data sheets and replaced with an anonymous participant identification number. Written consent forms were sealed and are being securely stored for five years, after which they will be destroyed.

3. Results

Demographic data indicated significant variability between individuals for sexual, romantic, and paternal characteristics (see Tables 4 & 5). Some participants failed to answer all of the demographic questions. Likewise, some participants reported difficulty in selecting the appropriate answer to a question; for example, some participants were sexually active with partners outside of their primary relationship (with or without their partner's knowledge).

Table 4. Behavioral/individual variables results.

Variable	Min	Max	Mean	(SE)
Age (years)	19	45	31.35	(1.09)
Number partners (#)	0	25	6.78	(1.06)
Future partners (#)	1	10	1.70	(0.32)
Rel. quality (DAS total)	78.00	137.00	112.02	(2.07)
Relationship care (IBM)	13	36	28.67	(1.08)
Relationship control (IBM)	1	31	9.98	(0.96)
Doll score (Baby Doll score)	0	100	65.11	(5.08)
Cry minutes (mins)	0	14	5.36	(0.61)

Table 5. Categorized behaviorial data results.

Variable	1	2	
Time kids (1=hi, 2=lo)	n=22	n=30	
Want kids (1=y, 2= n)	n=40	n=12	
Rel. status (1=single, 2=partner)	n=12	n=37	
Sex frequency (1=lo, 2=hi)*	n=26	n=13	
Origin	Newfoundland = 31	Canada = 11	Other = 8
Have kids	Infant = 11	Child(ren) = 12	Non-Fathers = 29

**Sex frequency – subjective assessment, 13 participants skipped this question*

3.1. Testosterone levels: Individual Differences

I started my analyses of T by running simple preliminary statistical tests of individual differences in T data as suggested by previous research findings (Gettler et al., 2011; Storey et al.,

2000; van Anders et al., 2012a). Testosterone data were analyzed as baseline (before experimental interaction), reactive (after experimental interaction), and change in T (reactive – baseline; positive value equals T increase, negative value equals T decrease). There were non-significant negative correlations between the scores on the baby doll test and the T levels (strongest correlation between the baseline in the video condition, $r_{36} = .28$, $P = .103$)

First, I tested for differences between baseline and reactive T levels in stages of fatherhood: fathers of young infants, fathers of older children, and non-fathers. There was significantly lower T in fathers of young infants than non-fathers in baseline T levels both before ($F_{2,43}=7.70$, $p<0.01$) and after the video session ($F_{2,43}=5.36$, $p<0.05$, Table 6). There were no significant differences between state (father vs. non-father) or stage (father of infant vs. older children) of fatherhood for the change in T during the video session. There were no significant differences between fatherhood status or stage in the baby doll session, but there was a trend for T to be lowest in fathers of infants, higher in fathers of older children, and highest in non-fathers.

Table 6. T levels (nmol/l; M±SE) in the video and baby doll sessions, including baseline, reactive, and the change in T across sessions, e=eta^2, F = father, NF = non father. Note that not all participants in the video session were also in the Doll session, hence the different Ns and means.

Variables – M(SE)	**F-Infants**	**F- Children**	**NF**	
Video Session	N=10	N=7	N=27	e, Power
Baseline T	**12.12 (0.87)[a]**	17.55(1.92)	**21.43(1.46)[a]**	**.27, .84**
Reactive T	**12.89(1.15)[a]**	18.07(2.12)	**20.94(1.10)[a]**	**.21, .70**
Change T	0.77(0.46)	0.52(0.93)	-0.49(0.32)	.10, .42
Doll Session	N=6	N=11	N=29	
Baseline T	15.26(1.97)	20.16(2.19)	21.33(1.46)	.07, .32
Reactive T	16.60(2.02)	19.05(1.52)	20.85(1.29)	.05, .23
Change T	1.34(0.61)	-1.11(0.91)	-0.48(0.50)	.07, .32

[a] the same letter in the same row indicates a significant difference $p<0.05$ (bold).

To compensate for the possibility that some of the participants were too young to have had children yet, but may have the personal and physiological disposition to do so, I compared all men who reported currently wanting to have children with those men who did not. There was a significant difference in the change in T levels during the video session for men who reported wanting children vs. those that reported not wanting children ($F_{1,44}=5.62$, $p=0.02$). Men who reported not wanting children experienced a greater decrease in T levels than men who reported wanting children (Table 7). All other tests relating desire to have children and T levels were non-significant.

Table 7. T levels (nmol/l; M±SE) for men who report wanting children vs. those who do not want children. Please note that 44 men out of the 52 total answered this particular question.

Variables – M(SE)	**Want Children**	**Do Not Want Children**
Video Session	N = 33	N = 11
Baseline T	18.22 (1.32)	20.13 (2.08)
Reactive T	18.54 (1.33)	19.01 (1.99)
Change T	**0.31 (0.30)[a]**	**-1.11 (0.54)[a]**
Doll Session	N=34	N=12
Baseline T	19.96 (1.31)	21.10 (2.18)
Reactive T	19.56 (1.07)	20.74 (2.00)
Change T	-0.40 (0.51)	-0.36 (0.51)

[a] indicates significant group differences in bold, $p<0.05$

I also compared T levels in the video session to reported time with kids. Men in the group that reported greater time with kids (>10 hours per week) showed significantly increased T levels over the course of the video session compared to men in the group that reported little time (<10 hours per week) with kids ($t_{42}= 2.25$, $p= 0.03$; Power .55, Fig. 2).

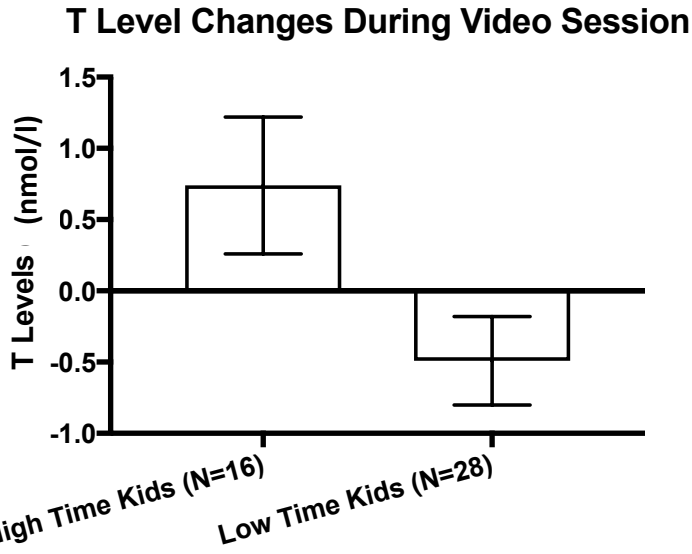


Figure 2. T levels (nmol/mL; M±SE) during video session by reported time with kids.

After completing preliminary testing of individual differences in T levels, I examined T levels further by comparing the relative fit of the hypothesized models with separate analyses for each proposed model: 3-Groups Model vs. RS-Partner/RS-Parent Model with CAG data for T levels in the video and baby doll sessions (see *GLM sections*).

3.2. AR gene – CAG repeats: Individual Differences

Analyses of CAG repeats of the *AR* gene included frequency distribution of the population (Fig. 3), analyses of the relationship between place of origin and CAGn, and inclusion of CAGn repeats as an independent variable in the multivariate GLM of T levels by Group-3 and RS-Partner/RS-Parent (see GLM sections). As shown in Figure 3, the most common number of CAG repeats were 21 (n=12), 24 (n=6), and 25 (n=6), out of a total of 46 successfully sequenced samples. Due to the distribution of CAG repeats, I conducted statistical tests with a mean split of the CAG number of repeats, as performed by previous authors (Campbell et al., 2007; Gettler et al., 2017). Additionally, I duplicated all statistical tests with CAGn as a continuous variable to tease apart any individual variation in the number of CAG repeats. After obtaining similar results

with all multivariate GLM tests of T levels and CAGn data by median (M=21.50) split as well as continuous variable data, I decided to report the results of the mean split data method as it was more straight-forward and easier to illustrate (as done in Gettler et al., 2017). However, my results suggest that further research regarding the specific number of CAG repeats may be of value to the field as the three men with 19 CAG repeats were consistently different from other genotypes on multiple behavioral categories. I found no significant differences in CAGn by place of origin ($F_{2,45}$=0.13, p=0.88). I also found no significant interaction between CAGn and place of origin as they relate to T levels in the video or the baby doll sessions.

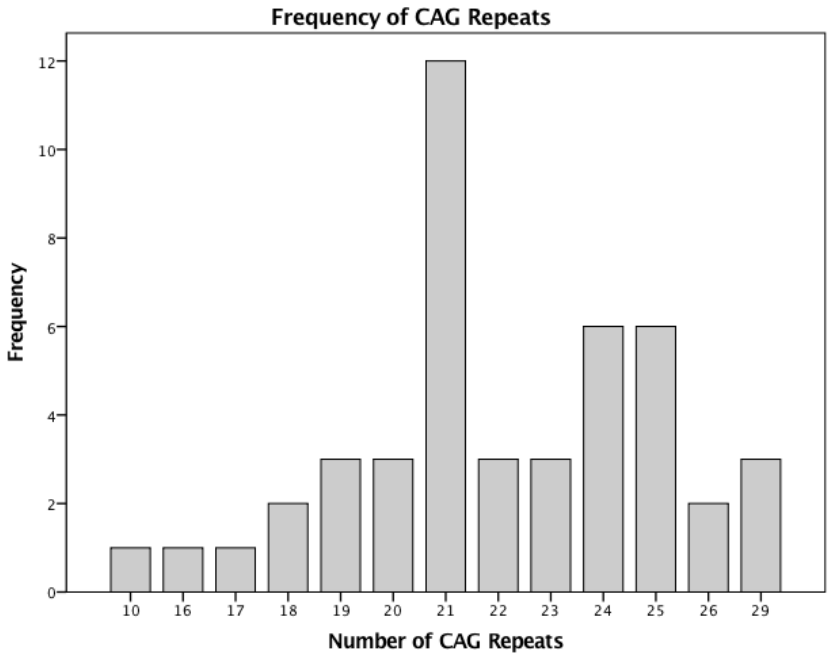


Figure 3. Frequency of CAGn (N=46, M=22.09, SE=0.51, Median=21.50, Mode=21.00)

I then performed preliminary testing of CAGn and demographic data by t-test to determine whether a greater number of CAGn is associated with lower relationship orientation. To test this hypothesis, I compared high (21-29) and low (10-20) CAGn with demographic data

for relationship characteristics. Whether tested by mean split or continuous variable definition, there was a significant difference in "relationship control" ($F_{11,38}$= 2.980, p=0.01), but not for "relationship status", "sex frequency", "number of sexual partners", "relationship care", or "relationship quality". Post hoc analyses indicated that men with fewer CAG repeats (10-20) reported that their partners were more controlling (IBM questionnaire, M=12.04 $\pm$ 0, SE=1.42) than men with more CAG repeats (M=7.06 $\pm$, SE=1.08; t_{37}=2.60, p=0.014, eta^2 = .23, Power = .81). Additionally, when treating the number of CAG repeats as a continuous variable, I found that CAG repeats were independently related to behavioral characteristics, i.e., there was no additive effect of the number of CAG repeats. Specifically, individuals with 10, 19, 21, or 23 CAG repeats reported experiencing more controlling behavior from their partner.

3.3. 3-Group Model: Behavioral Analyses

There were no significant differences among the three reproductive groups for demographics, including age ($F_{2,51}$=0.51, p=0.61), weight ($F_{2,51}$=1.98, p=0.15), height ($F_{2,51}$=1.77, p=0.18), or hours of sleep ($F_{2,51}$=0.18, p=0.83).

K-means cluster analyses with three categories generated groups of behaviors with significant differences between hypothesized reproductive strategies (3-Groups: Bachelor, Provider, and Direct Father) as indicated by one-way ANOVA and Fisher's Exact Test. There were significant differences between the three reproductive strategies for "Time with Kids" (Fisher's Test: P_A=0.022); "Doll Score" ($F_{2,44}$=121.81, p<0.01); "Cry Mins" ($F_{2,44}$=33.76, p<0.01); "Number Partners" ($F_{2,44}$=3.80, p=0.03); and "Relationship Care" ($F_{2,41}$=3.33, p=0.046, eta^2 = .15, Power = .55). Review of the patterns of individual differences resulted in three distinct groups that map onto the hypothesized groups of Bachelor, Provider, and Direct Father (Table 8), with the exception of "Time with Kids'. Specifically, men in the Provider group reported spending more time with children than men in the Direct Father group. However, both types of fathers reported spending more "Time with Kids" than men in the Bachelor group.

There were significant differences between all three groups for “Doll Score”, with higher scores indicating more effective care and significantly fewer cry minutes. Men in the Direct Father group showed higher scores than those in the Provider group, with men in the Bachelor group showing the lowest doll scores (Table 8). However, men in the Provider group were not significantly different than men in the Bachelor group for doll score. Men in the Bachelor group reported a higher number of previous sexual partners than men in the Provider and Direct Father groups ($F_{2,44}$= 3.80, p=0.03). There was no significant difference between the number of partners reported by men in the Provider and Direct Father groups. Despite a significant ANOVA result, the corrected Bonferroni test for “Relationship Care” resulted in a non-significant difference among the groups (Table 8).

Table 8. ANOVA results of demographic data for hypothesized descriptions of 3-Groups – N=52, (M±SE)

Variables	**Bachelor (N=4)**	**Provider (N=13)**	**Direct Father (35) eta^2, Power**
Age (years)	30.00 (3.54)	33.46 (2.03)	30.71 (1.38)
Have Kids	0 (0%)	8 (62%)	16 (46%)
Time with Kids*	0/4[a]	**9/4**[a]	**13/22**[a]
Doll Score	**0.00**[a] (0.00)	**33.08**[a] **(2.37)**	**87.34**[a] (2.88) .85, .80
Cry Mins (mins)	**11.50**[a](0.87)	**8.83**[a] (1.07)	**3.07**[a] (0.38) .62, 1.0
% Partnered	0%	92%	65%
No. Past Partners	**15.67**[a](5.21)	**3.73**[a] (1.15)	**7.00**[a] (1.30) .15, .61
No. Future Partners**	2.67 (1.67)	1.08 (0.08)	1.88 (0.47) .05, .13
Relationship Quality	126.02 (3.53)	112.40 (3.12)	110.26 (2.75) .10, .38
Relationship Care	32.00 (3.06)	32.25 (1.47)	26.70 (1.40) .15, .55
Relationship Control	9.98 (2.08)	10.58 (0.93)	9.81 (1.43) .005, .07

[a] same letter in the same row indicates a significant difference between groups, $p < 0.05$

* "time with kids" as a scale of time spent with children: 1.0=>10 hours, 2.0=<10 hours per week

** Future partners based on individual estimate of the number of future sexual partners.

3.4. RS-Partner/RS-Parent Model: Behavioral Analyses

There were no significant differences in demographic results for RS-Partner/RS-Parent including: age (t_{50}=1.70, p=0.09), weight (t_{50}=0.24, p=0.09), height (t_{50}=-0.18, p=0.86), or hours of sleep (t_{50}=0.180, p=0.09).

Following analyses of the three group reproductive strategies, I isolated partner behaviors and ran additional K-means cluster analyses selecting for two hypothesized groups: High vs. Low Partner Orientation. Results of the K-means clustering indicated significant differences in partner

behaviors for the two groups. High partner orientation men reported significantly fewer past sexual partners than low partner orientation men ($F_{1,43}$=11.50, p<0.01). Likewise, high partner orientation men predicted that they would have fewer future partners than low orientation men ($F_{1,38}$=6.05, p=0.02). Overall quality of relationship as measured by the "dyadic total" score was significantly higher for high partner orientation men than for low partner orientation men ($F_{1,40}$=75.25, p<0.01). High partner orientation men reported greater care in their relationships than low partner orientation men ($F_{1,40}$=3.24, p=0.08). Men with high and low partner orientation did not differ in the amount of control dynamics they experienced in their relationships by their partner ($F_{1,40}$=0.21, p=0.65; Table 9).

Table 9. Results (M±SE), demographic data for hypothesized descriptions of RS-Partner, e =eta^2

Variables	**Low Partner (N=15)**	**High partner (N=36)**	**eta^2, Power**
	N=12, 80% Partnered	N=25, 70% Partnered	
No. past partners	**11.85 (1.74)[a]**	**4.72 (1.14)[a]**	.21, .85
No. Future partners[*]	**2.83 (0.93)[a]**	**1.21 (0.18)[a]**	**.14, .66**
Rel. Quality (DAS Tot.)	**96.01 (2.71)[a]**	**119.19 (1.32)[a]**	**.65, 1.0**
Relationship care (IBM)	**25.85 (2.26)[a]**	**29.93 (1.14)[a]**	**.08, .40**
Relationship control (IBM)	10.40 (1.87)	10.09 (1.13)	.005, .07

[a] significant difference between groups in bold, p <0.05

[*] future partners based on individual estimate of the number of future sexual partners

K-means of parenting behaviors selecting for two hypothesized groups: High vs. Low Offspring orientation (high vs. low nurture) men resulted in significant differences between groups. However, these differences were difficult to map onto the hypothesized groups because there were fewer fathers in the High Nurture group (34%) than in the Low Nurture group (59%).

Additionally, men in the High Nurture group reported spending less time with children than men in the Low Nurture group (t_{50}=2.15, p=0.04, Table 10). However, men in the High Nurture group scored significantly higher on the RealCare Baby doll interaction than men in the Low Nurture group (t_{43}=12.67, p=0.00). Likewise, men in the High Nurture group provided more successful care, as defined by fewer 'cry' minutes, than men in the Low Nurture group (t_{43}=-7.82, p< 0.01; Table 10). After consideration of clustering results, I have defined the High and the Low Nurture groups by the relative success of the "Doll Score" and "Cry Mins" rather than the parental status or time spent with children, as the "Doll Score" and "Cry Mins" are quantitative data rather than qualitative and therefore less subjective. Future research should consider alternative means of evaluating the breadth and variability of nurturing care.

Table 10. Results of demographic data for hypothesized descriptions of RS-Parent – M(±SE)

Variables	**High Nurture (N=30)**	**Low Nurture (N=22)**	**eta^2, Power**
Number of fathers	N=10, 34% Fathers	N=13, 59% Fathers	
Time with kids*	**1.70 (0.09)[a]**	**1.41 (0.01)[a]**	**.09, .53**
Doll score	**87.34 (2.80)[a]**	**24.81 (4.10)[a]**	**.79, 1.0**
Cry mins	**3.07 (0.38)[a]**	**9.50 (0.87)[a]**	**.59, 1.0**

[a] significant difference between groups in bold, p <0.05

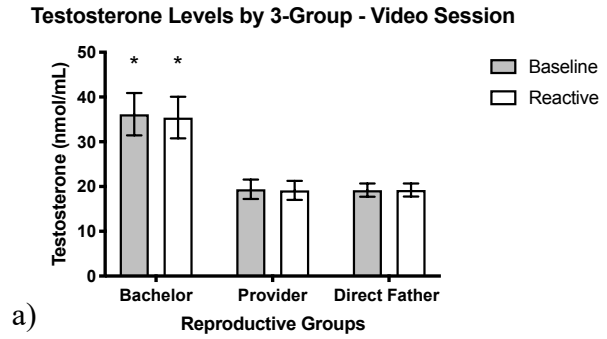
* "time with kids" as a scale of time with children: 1.0 ≥ 10 hours, 2.0 ≤ 10 hours per week

3.5. GLM of the effect of 3-Group model and CAG repeats on T levels

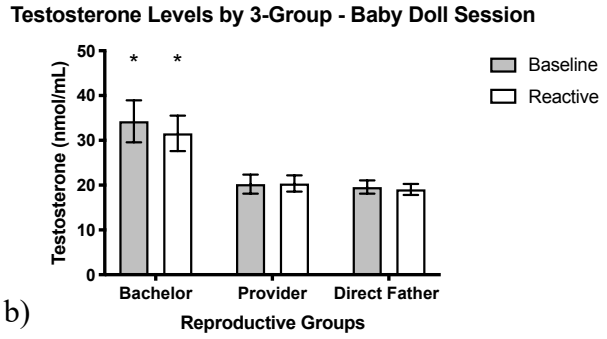
I performed GLM analyses of the two independent variables of group membership and high or low number of CAG repeats on the dependent variable T levels to determine model fit. Analyses of the 3-Group model indicated a main effect of group with men in the Bachelor group demonstrating significantly higher T than men in the Direct Father or the Provider groups, both

before ($F_{2,27}$=5.98, p<0.01, eta^2=.31, Power= .83) and after ($F_{2,27}$=5.69, p<0.01, eta^2=.30, Power=.80) the video session (Fig. 4a). Similarly, for the baby doll session, men in the Bachelor group had significantly higher T than men in the Direct Father group or the Provider group both before the interaction ($F_{2,27}$= 4.49, p=0.02, eta^2=.26, Power=.71) and after the interaction ($F_{2,27}$= 4.57, p=0.02, eta^2=.25, Power=.72; Fig. 4b). There were no significant group differences in the change in T levels from before to after both the video and baby doll sessions. There were no significant main effects for the second independent variable, number of CAG repeats, and the three groups on baseline, reactive, or change in T levels in either the video or the baby doll session (eta^2 =.002-.030; Power =.06 -.18).

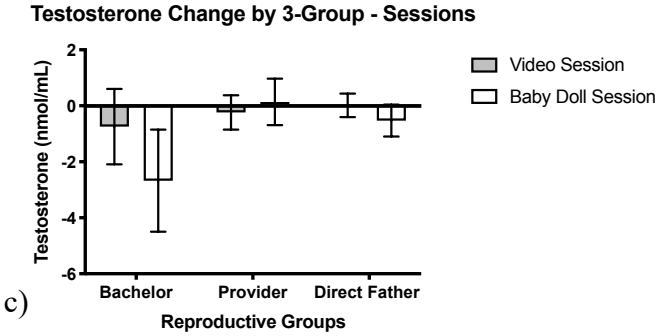
There was also a significant interaction between the 3-Groups and the number of CAG repeats for the change in T levels during the baby doll session ($F_{2,27}$=4.250, p=0.025, eta^2=.24, Power=.67; Fig. 4c), but not the video session. The interaction was due to high T levels and a large decrease in the single participant in the bachelor group with few repeats, compared to men with few repeats in the other two groups, and thus must be treated with extreme caution.



a)



b)

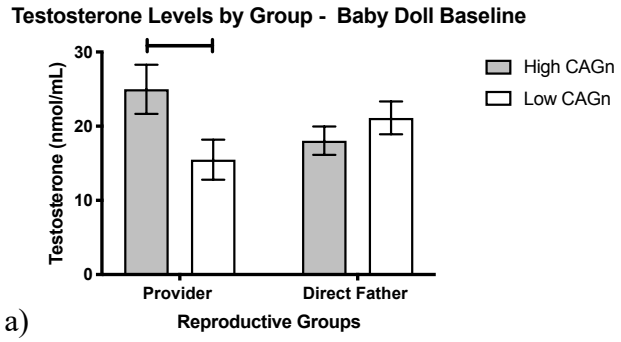


c)

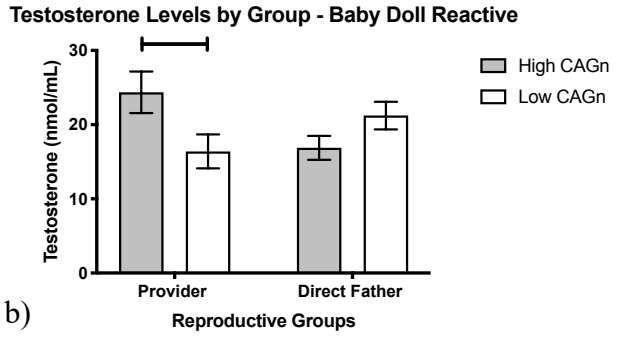
Figure 4. T levels (nmol/mL; M±SE) during by 3-Group membership for video (a), baby doll (b), and change during sessions (c).

* groups are significantly different than all other groups in the same session.

Because of the extremely small sample size in the Bachelor group, I re-ran the GLM of 3-Group Model and CAG repeats with only data from the Provider and Direct Father groups. In the baby doll session, men in the Provider group with high CAGn had significantly higher T levels, for both baseline ($F_{1,27}$= 5.87, p=0.02, eta^2=.18, Power=.65) and reactive T ($F_{1,27}$=7.92, p<0.01; eta^2= .23, Power= .77), than did men in the Provider group with low CAGn (Fig.5). Men in the Direct Father group did not differ significantly in their T levels by CAGn. There were no significant differences between groups in the video session.



a)



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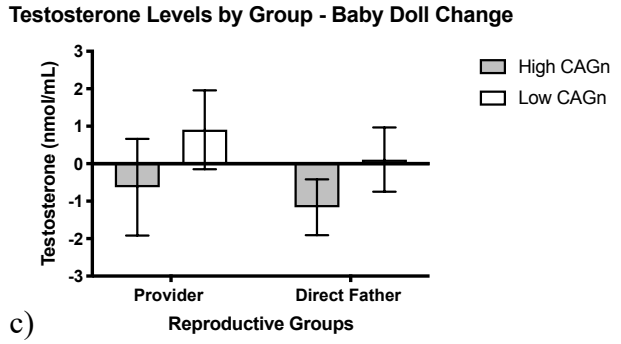

c)

Figure 5. T levels (nmol/mL; M±SE) during by 3-Group membership and CAGn for video (a), baby doll (b), and change during sessions (c).

* brackets indicate significant differences between groups

3.6. GLM of the effect of RS-Partner/RS-Parent model and CAG data on T levels

There were no significant main effects of RS-Partner, RS-Parent, or CAG repeats on T levels, both before or after testing in either the video or baby doll sessions. There was, however, an interaction between RS-Partner and RS-Parent in the change in T levels during the baby doll session ($F_{1,26}$= 4.61, p=0.04, eta^2=.15, Power=.50; Table 11). However, post-hoc analyses of the group differences in the RS-Partner/RS-Parent interaction by one-way ANOVA or simple main effects yielded non-significant results, likely due to small sample size.

Table 11. T level (nmol/mL; M±SE) changes during the baby doll interaction by RS-Partner/RS-Parent model.

Reproductive Strategies	**N**	**Mean (SE)**
High Partner/High Parent	17	-0.27(0.47)
High Partner/Low Parent	13	0.17(0.38)
Low Partner/High Parent	7	0.52(0.73)
Low Partner/Low Parent	7	-0.45(0.88)

There was also a significant interaction between RS-Parent and CAG repeats in T levels following the baby doll session ($F_{1,26}$= 4.47, p=0.04, eta^2=.15, Power=.53; Fig. 6). It appears that the interaction is due to differences in men with a greater number of CAG repeats in the two parental categories; however, post-hoc analyses of the group differences in the RS-Parent and CAG repeats interaction by one-way ANOVA or simple main effects yielded non-significant results. There was no significant interaction between RS-Partner and CAG repeats for any of the analyses.

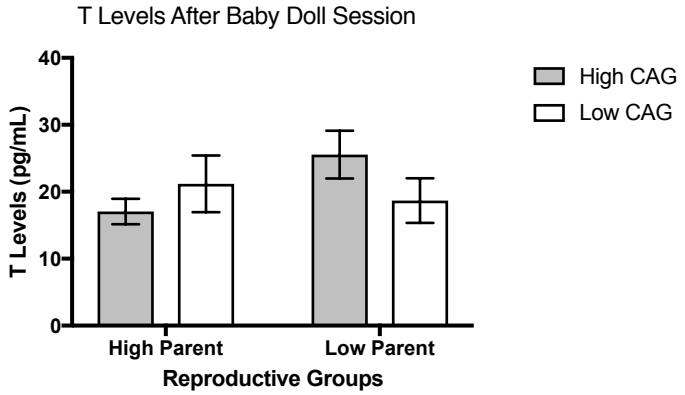

Figure 6. T levels (nmol/mL; M± SE) after the baby doll session by RS-Partner/RS-Parent model and number of CAG repeats.

Lastly, analyses of CAG data as a continuous variable in the GLM of RS-Partner/RS-Parent and CAG repeats on T levels produced significant differences between men with 19 CAG repeats and men with several other number of CAG repeats (Table 12). Specifically, there were significant differences between T levels and the number of CAG for reactive T in the video session ($t_{37} = -2.16$, $p=0.04$), baseline T in the baby doll session ($t_{38} = -2.71$, $p=0.01$), and reactive T in the baby doll session ($t_{38} = -2.28$, $p=0.03$), but not the other sessions. Despite the significant differences due to 19 CAG repeats and other repeats, omitting 19 CAG repeats from the previous high/low repeats did not change the results.

Table 12. T levels (nmol/l; M±SE) for 19 CAG vs. all other numbers of CAG repeats.

Session	19 CAG (n=3)	Others (n=36)
Video session		
Baseline T	27.02 (8.16)	18.18 (1.16)
Reactive T	**27.58 (7.31)[a]**	**18.16 (1.15)[a]**
Change T	0.56 (1.13)	-0.02 (0.30)
Baby Doll session		
Baseline T	**33.06 (7.67)[a]**	**19.39 (1.11)[a]**
Reactive T	**29.13 (4.13)[a]**	**19.10 (0.98)[a]**
Change T	-3.94 (3.54)	-0.29 (0.40)

[a] significant difference between groups in bold, $p < 0.05$

4. Discussion

Significant findings indicate that there is evidence of alternative mating strategies in this population of men. Specifically, the 3-Group model supported a tendency for short-term mating strategies and elevated T for men in the Bachelor group. Men in the Provider group displayed mixed strategies and variable T levels. Men in the Direct Father group displayed low and stable T levels and long-term mating behaviors. Additionally, men with lower average number of CAG repeats report experiencing more control from their partners, and men with specific low numbers of CAG repeats (10, 19, 21, & 23) had higher T levels than other men.

4.1. Demographic tests of Testosterone levels

There were no significant differences between individuals for age, weight, height, or hours of sleep and T levels. Previous literature has found mixed results for differences in T levels by age, weight, height, and hours of sleep. However, the general trend in the literature is that men between adolescence and advanced age, at a healthy weight, and experiencing an average amount

of sleep will express similar T level patterns with little diurnal variation (Axelsson et al., 2012). As this population was comprised of men fitting these general criteria, these findings were as expected.

Overall, fathers of young infants had significantly lower T (baseline and reactive) than non-fathers during the romantic video session. T levels of fathers of older children were not significantly different from the other two groups but the large effect size suggests that this result should be re-examined with a larger sample size. These results support previous literature indicating a strong basis for the claim that, in general, fathers have lower T levels than non-fathers (Alvergne et al., 2009; Fleming et al., 2002; Gettler et al., 2011; Storey et al., 2000), but that the effect of fatherhood on T levels is strongest during the first year after the birth (Gettler et al., 2014; Gettler et al., 2011; Mascaro et al., 2013; Weisman et al., 2014). T levels in the baby doll session were not significantly related to fatherhood status for either baseline or reactive T, despite showing the same trend for lower T in fathers than non-fathers.

Men who reported a desire to have children had a different pattern of change in T levels following the video session than men who reported no desire to have children. Specifically, men who reported wanting children experienced a greater increase in T levels than men who did not want children. Interestingly, men who reported spending more time with children also had a greater increase in T during the video session than men that reported spending less time with children. It is possible that men that are currently utilizing a mating strategy for the purposes of producing additional children may respond to romantic stimuli with the partner with increased T.

There are additional factors to consider when evaluating these results: the content of the videos used in this study, and the impact that the study's description may have had on the participants. While one of the videos shown during the video session was mildly sexual, both videos depicted the beginning of a monogamous romantic relationship. It is possible that the videos were more physiologically stimulating to those men interested in a long-term relationship

and having a child as opposed to men not interested in having a child. In contrast, a related study found that men who were more interested in babies had less of a T level increase when viewing erotic video stimulation than men who reported less interest in babies (Zilioli et al., 2015). The authors suggested that the sexual content of the romantic interaction may have affected the men's hormonal response. It is important to note that the participants in the Zilioli et al. (2015) study were alone when viewing the erotic video, rather than with their partner, as in this study. The impact of viewing a romantic, rather than erotic, video coupled with the expressed parental inclination of the study description during the consent process may have influenced the participant's experience of the video session. Results of this study and Zilioli et al. (2015) suggest that a man's disposition as indicated by the type and intensity of their interest in the potential mate, rather than their parental status, may inform their hormonal response to relationship stimuli. In summary, these results suggest that T level changes are driven by a response to the partner rather than the "baby".

4.2. Analyses of CAG repeats

The most common genotype was 21 CAG repeats, with the next most common genotypes being 24 and 25 CAGn. These data support previous research that indicates an average number of CAGn is between 20-22 in most populations, with a range of 8 to 37 CAGn (Butovskaya et al., 2015; Kawasaki et al., 1999; Panizzon et al., 2017). Previous research has employed various methods of analyzing for CAG repeats. For example, Campbell et al. (2007) used cut-off points published in previous literature (i.e., 20 CAGn), while Simmons and Roney (2011) split their CAGn data by median value. Based on this inconsistency in the literature, I began the analyses of CAGn by comparing number of repeats to place of origin, both by mean and median split and as a continuous variable. Results indicated no differences in CAGn by place of origin (i.e., Newfoundland, Canada, Other) regardless of analyses method.

After evaluating possible relationships between CAGn and demographic variables, the only significant relationship was between number of CAGn and reported feelings of control in the relationship. Men with fewer CAGn repeats reported experiencing more controlling dynamics in their relationship by their partner than men with more CAGn repeats, a result that was compatible with previous research indicating greater competitive (Eisenegger et al., 2017) and aggressive tendencies in men with fewer CAGn (Butovskaya et al., 2015). For clarification, the term "controlling dynamics" refers to conflict within the relationship related to control of the other relationship partner by either partner. Likewise, analyses of CAGn as a continuous variable indicated that all of the men that reported experiencing greater control dynamics in their relationship by their partner had CAGn of 23 or fewer.

4.3. Behavioral Analyses: 3-Group model

K-means clustering for 3-Groups indicated significant differences between groups for time with kids, RealCare doll score, number of past sexual partners, and reported care in the relationship. As predicted, baby doll scores were highest in men in the Direct Father group, moderate scores in men in the Provider group, and low in men in the Bachelor group. Care in the relationship was high for men in the Provider group, moderate for men in the Direct Father group, and low for men in the Bachelor group. However, time with kids and number of sexual partners resulted in surprising differences between groups (discussed below). There were no demographic differences between groups.

An interesting result of the 3-Group analyses of behavioral data was the number of sexual partners reported for each of the three groups. As expected, men in the Bachelor group reported the highest number of past sexual partners while men in the Provider and Direct Father groups reported significantly fewer. Though not a significant difference, men in the Direct Father group reported higher than expected number of past partners, possibly due to lack of monogamous

partnerships. Future research should examine the sexual history of men in different reproductive strategies to better understand these data.

It is important to note the lack of difference in age between groups, which rules out the possibility of older men having a higher number of sexual partners due to simple opportunity and passage of time. Many research articles have focused on the trait differences between men such as dads vs. cads (Cashdan et al., 1993; Durante et al., 2012; Kruger & Fisher, 2005), or slow vs. fast reproductive strategies (Brumbach and Figueredo, 2009; Vladas et al., 2011) to explain a multitude of characteristics including sexual behavior. For example, one study of male reproductive behavior focused on men as involved fathers, providers, deadbeat dads, or paternity free men (Marks and Palkovitz, 2004). The authors compared and contrasted trends in the relative proportions of hypothesized categories between the 1920s/1930s and the early 2000s, finding a modern increase in men remaining paternity-free based on census data. Similar to these findings, a large portion of men in this study reported a desire not to have children. However, research also suggests that men's reproductive behavior can change over time and in response to their current reproductive context. For example, men can adjust their reproductive behavior as their desire for sexual novelty has been satiated (Puts et al., 2015). In this study, the higher numbers of past sexual partners reported by men in the Direct Father group than men in the Provider group may be due to having satiated their desire for sexual novelty and fully transitioned from mate seeking to offspring care as described in Gettler et al. (2011).

One of the overall strengths of this study was quantifying offspring care success through the use of the RealCare baby doll. van Anders et al. (2012b) manipulated participant's ability to care for the RealCare baby doll in order to examine T levels relative to the effectiveness of male nurturance. They found that men able to provide effective infant care experienced a relative decrease in T levels following baby doll care compared to men unable to provide effective care, who experienced a relative increase in T levels (van Anders et al., 2012). While the previous

study addressed the responsive nature of T to an acute infant care interaction, it did not focus on the individual differences in the men participating in the study. Since my study utilized behavioral differences to characterize the reproductive strategies of individuals, I was able to interpret the differences in the effectiveness of the nurturant care provided to the RealCare doll as a function of the individual's reproductive strategy, current relationship, and parenting context. I interpret the effective nurturant care scores for men in the Direct Father group as indication that they are predisposed to respond to parental tasks more effectively than men in the Provider or Bachelor groups. As expected, men in the Provider or the Bachelor groups did not demonstrate familiarity or comfort with the RealCare doll. Previous research has shown that characteristics such as family history, parenting attitudes, attachment style (Mckay, 2016) and relationship quality (Kuo et al., 2016) can influence the responsiveness of the caregiver. Additionally, recent training and experience with infants has been show to influence effective caregiving (Doherty et al., 2006).

While my predictions about time with kids were supported for men in the Bachelor group, results did not support my predictions for men in the Provider group who reported spending more time with children than men in the Direct Father group. It is important to note that a greater number of men in the Provider group reported having children than men in the Direct Father Group, which could be due to the young age of many of the participants during the study. There may also be a multitude of family dynamics at play that can affect time spent with children. For example, custody agreements, geographic proximity to the children, and the subjectivity of self-report can all affect reported time spent with children. Men in the Provider group may be estimating their time with children by referencing their time available outside of working hours, or by comparison to their social peers. In contrast, men in the Direct Father group may be estimating their child-care efforts by comparing themselves to that of the primary caregiving parent or other primary caregivers. Future research should consider more objective measures of time spent with children, including spousal report or daily logs.

4.4. Behavioral Analyses: RS-Partner/RS-Parent model

Men in the High Partner group reported fewer past and anticipated fewer future sexual partners than men in the Low Partner group, though these differences were not related to differences in T levels. Ostovich and Sabini (2004) found that characteristics of sociosexuality, as measured with the Sociosexuality Inventory (a measure of restrictiveness in sexual attitudes), were the best predictors of number of sexual partners. Likewise, a study of multiple individual characteristics found that high T, high sensation seeking, and disinhibition of sexual cultural norms were all predictors of a higher number of sexual partners for men (Bogaert and Fisher, 1995). Similarly, men in the High Partner group in this study reported higher relationship quality than men in the Low Partner group, supporting previous findings of a negative relationship between relationship quality and T (Edelstein et al., 2014). Results for reported relationship care and control were not significant, possibly due to small sample size. However, the balance between care and control in a relationship is worth study as that balance is indicative of equality and reciprocity between partners.

Interpretation of parental orientation is difficult when considering the unexpectedly high number of fathers and the high amount of reported time with kids in the Low Nurture group. The apparent paradox in the results of RS-parenting with regard to the presence of few fathers in the High Nurture group and low time with kids, yet high doll score and low cry mins in the doll session may be explained by the differences in the accuracy of the doll as a proxy for human infant care giving (see van Anders et al., 2012b). More likely, however, time with kids may not always be indicative of a man's desire to interact with children as many social factors (i.e., availability of a suitable partner, access to children due to custody agreements, infertility, etc.) may inhibit a man from spending as much time with children as desired. Alternatively, the RealCare baby doll may not be the best predictor of familial behavior in men outside of the laboratory context. However, for the purposes of this study, parental orientation results fit with

my predictions, as the RS-Partner/RS-Parent model was a good fit for the variables, despite the small sample size.

4.5. Two models in relation to CAG repeats

In the 3-Group model, men in the Bachelor group had higher T than men in Direct Father and Provider groups before and after the video session and baby doll sessions, as predicted but there were no main effects associated with having a greater or fewer number of CAG repeats. In the analysis of the two groups (omitting the bachelor group with its small sample size), men in the Provider group with a high number of CAG repeats had significantly higher testosterone levels than men in that group with a low number of repeats. There were no significant main effects for number of repeats and group for the RS-Parent/RS-Partner analyses and the resulting significant interactions were difficult to interpret. Further study with a larger sample size is needed.

4.6. GLM RS-Partner/RS-Parent model and CAG on T levels

In the RS-Partner/RS-Parent model, groups varied in their T responses to the video session, suggesting that partnering and parenting behaviors are characterized by differentially affected responses to romantic stimuli. Due to the non-significant results in the post-hoc analyses of the interaction between the RS-Partner and RS-Parenting, no concrete conclusions can be drawn from these data. However, previous research has shown that men reporting high levels of commitment and relationship satisfaction had lower baseline T than men who reported lower commitment and satisfaction in their relationship (Edelstein et al., 2014). Unfortunately, Edelstein et al. (2014) only examined aspects of intimate partner relations without inclusion of parenting interactions. Further study of this interaction could clarify interdependence of partnering and parenting.

Although the significant interaction between parenting and partnering strategies did not withstand post-hoc testing due to the small sample size, it did suggest that future studies should compare men with mixed strategies (i.e., high partner/low parent or low partner/high parent) for

increases in T levels, and men with direct strategies (high partner/high parent or low partner/ low parent) for decreases in T levels when caring for a baby doll. This potential result brings into question a man's motivation to spend time with children (i.e., for the purpose of being close to the child, or to retain mate access), as well as the quality of that interaction. Previous research had shown T decreases in men providing significant amounts of childcare; however, more recent research has differentiated between time spent providing nurturing care, rather than casual interaction with an infant, influences the T response to caregiving interactions (see van Anders et al., 2012b). I propose that this context of the interaction, as well as the currently employed strategy will work together to fine-tune a man's physiology. Unfortunately, I was unable to assess the subjective experience of the men during the video session as the post-interaction questionnaire did not include detailed questions about the experience of the video sessions. Future research should include assessment of the man's interest in and enjoyment of time spent with children when interpreting T levels, as well as how they felt when watching the romantic videos with their partner.

The non-significant results of the RS-Partner/RS-Parent in the baby doll session could be due to the model itself, the efficacy of the RealCare baby doll, or the K-Clustering of the baby doll session data. As described previously, RS-Parent was defined by the doll score and cry mins only, as the estimated amount of time with children and proportion of fathers in each group were in contradiction. Future studies would benefit from additional data related to fathering behaviors, such as partner report of time with children, questionnaires related to participant's experience of time with children, subjective report of how much the participant enjoys caring for children, etc.

In summary, despite interesting and significant results in the behavioral analyses the overall value of the RS-Partner/RS-Parent Model is lower than the 3-group model due to complicated results for T levels and CAG repeats. Further analyses of the RS-Partner/RS-Parent odel with other hormones and their genetic counterparts (i.e., oxytocin and markers for oxytocin

receptor genes, vasopressin and markers for vasopressin receptor genes – see Chapter 2) may yield different and possibly more productive results for this model.

4.7. Overall Considerations

A point of interest was that inclusion of CAG data as a continuous variable in both models produced significant differences between men with 19 CAG repeats and other numbers of CAG repeats. It should be noted that this is a preliminary result as there were three men with 19 CAG, however these men did have significantly higher baseline and reactive T across sessions. As mentioned previously, authors have analyzed CAGn by multiple methods including mean/median/SD split (for examples, see Butovskaya et al., 2015; Campbell et al., 2007; Celec, 2013; Gettler et al., 2017). The lack of a consistent convention for analyzing CAG repeat polymorphisms may mask some of the differences between specific genotypes. I suggest that future studies look at the specific number of CAG repeats in more detail to identify subtle physiologically functional differences, aside from the transcription activity of T, which may influence reproductive behavioral ecology.

One last note on the use of the RealCare baby doll in this study is that after examining the T levels in the baby doll session, I went back to the session notes for the baby doll sessions. Many of the men commented on their post-interaction questionnaires that the RealCare baby "felt real" for the first few minutes, but following that time they began to feel that the doll "was like a video game". If some of the participants were experiencing the baby doll as "robotic" or "game-like", their physiology may not have been responding to infant care stimuli as much as competitive stimuli, calling into question the efficacy of the doll as a proxy for an actual infant. Previous research has shown that competitive stimuli often increase T levels, both prior to and immediately following the task (Carré and Olmstead, 2015). In addition, the perception of effective contribution to the competition can affect T levels, such that if a man feels unprepared to effectively compete, he may experience a decline in T regardless of the ultimate competition

outcome (Gonzalez-Bono et al., 1999). Future research should consider a training period prior to experimental interaction to assure that participants are comfortable with the RealCare baby doll and perform a more extensive comparison of men's responses to the doll and to a real baby.

5. Conclusions

This study is the first to evaluate reproductive behavior, endocrinology, and genetics within the context of differential models of human reproductive strategies. My findings support previous research indicating that men's sociosexual behavior is influenced by genetics (Zitzmann, Nieschlag, 2003; Gettler et al., 2017), endocrine profile, and their current social context (van Anders et al., 2015; Edelstein et al., 2014; van Anders et al., 2013). My results extend current understanding of the area by proposing conceptual models that represent the interdynamic nature of the relationships between genetics, endocrinology, and social behavior across individual differences and contextual effects.

The 3-Group Model surpassed the RS-Partner/RS-Parent Model by yielding significant group differences for partnering characteristics. The surprisingly high numbers of past sexual partners for men in the Direct Father group suggest that men in the Direct Father group have successfully satiated their desire for sexual novelty and transitioned to nurturant partners in both partnering and parenting. However, one strength of the RS-Partner/RS-Parent analysis is that it allowed a clearer separation of partnering characteristics from parenting characteristics, resulting in a distinction between individuals that were as closer to my predictions for previous sexual experience. These analyses suggest that investment in partnering and parenting is determined individually and is specifically dependent on the man's personal and physiological disposition.

To clarify these claims, I suggest that interest in partnership may be the gateway to interest in parenting for men, as interacting with the partner is the primary intimate relationship in these men's lives and, therefore, is the largest source of T impact. Specifically, the high T levels of men in the Bachelor group, followed by the moderate T levels of men in relationships with

children indicate the transitional effects that relationships and parenting can have. Additionally, significant changes in T levels during the video sessions rather than in the baby doll sessions provide further support for the effect of the partner on male reproductive strategies. Further examination of these processes may aid in understanding marital and parenting relationships and inform societal expectations that could support healthier sexual behavior and avoid the negative effects of divorce and inadequately nurturing paternal care. Similarly, additional analyses of the 3-Group Model in conjunction with cortisol levels will allow further examination of this relationship (see Chapter 3).

Study limitations include aspects of data collection that proved to be more complicated than originally expected. For example, definition of "relationship" was problematic in this study as men reported conflicting information about their relationship status. Future research should define "relationship" operationally (i.e. live with my partner, share household responsibilities, parent together, have sexual intercourse regularly with only one partner, etc.). Likewise, reported time spent with children resulted in unexpected patterns in the behavioral groups. Future studies should operationally and quantifiably define time spent with kids (i.e. sole caregiver for x hours per day, secondary caregiver for x hours per day, in the presence of and interacting with the child x hours per day, etc.).

Lastly, the use of the RealCare baby doll produced useful quantifiable data regarding men's behavioral and physiological response to infant stimuli. However, verbal reports by participants about the doll feeling like "a video game" or "feeling unreal" brings into question the validity of using the doll to measure paternal motivation and competence. To my knowledge, there has not been a validation study of the physiological effect of the RealCare dolls. A controlled comparison of T levels in fathers taking care of their infant compared the RealCare Infant should be carried out to determine whether the physiological effects are similar.

References

Alvergne, A., Faurie, C., Raymond, M., 2009. Father-offspring resemblance predicts paternal investment in humans. Anim. Behav. 78, 61–69. https://doi.org/10.1016/j.anbehav.2009.03.019

Axelsson, J., Ingre, M., Åkerstedt, T., Holmbäck, U., 2005. Effects of acutely displaced sleep on testosterone. J. Clin. Endocrinol. Metab. 90, 4530–4535. https://doi.org/10.1210/jc.2005-0520

Beilin, J., Ball, E.M.A., Favaloro, J.M., Zajac, J.D., 2000. Effect of the androgen receptor CAG repeat polymorphism on transcriptional activity: Specificity in prostate and non-prostate cell lines. J. Mol. Endocrinol. 25, 85–96. https://doi.org/10.1677/jme.0.0250085

Bogaert, A.F., Fisher, W.A., 1995. Predictors of University Men's Number of Sexual Partners. J. Sex Res. 32, 119–130. https://doi.org/10.1080/00224499509551782

Booth, A., Dabbs, J.M., 1993. Testosterone and men' s mariages. Soc. Forces 72, 463–477.

Booth, A., Johnson, D.R., Granger, D.A., 2005. Testosterone, marital quality, and role overload. J. Marriage Fam. 67, 483–498. https://doi.org/10.1111/j.0022-2445.2005.00130.x

Burnham, T.C., Chapman, J.F., Gray, P.B., McIntyre, M.H., Lipson, S.F., Ellison, P.T., 2003. Men in committed, romantic relationships have lower testosterone. Horm. Behav. 44, 119–122. https://doi.org/10.1016/S0018-506X(03)00125-9

Butovskaya, M.L., Lazebny, O.E., Vasilyev, V.A., Dronova, D.A., 2015. Androgen Receptor Gene Polymorphism , Aggression , and Reproduction in Tanzanian Foragers and Pastoralists. PlosOne 8–11. https://doi.org/10.1371/journal.pone.0136208

Campbell, B.C., Gray, P.B., Eisenberg, D.T.A., Ellison, P., Sorenson, M.D., 2007. Androgen receptor CAG repeats and body composition among Ariaal men. Int. J. Androl. 32, 140–148. https://doi.org/10.1111/j.1365-2605.2007.00825.x

Carre, J M, Geniole Shawn N., Ortiz Brian M., Videto Amber, B.P.L., 2016. Exogenous Testosterone Rapidly Increases Aggressive Behavior in Dominant and Impulsive Men: Testosterone modulates aggression in Dominant/Impulsive Men. Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2016.06.009

Carre, J.M., Olmstead, N.A., 2015. Social Neuroendocrinology of Human Aggression: Examing the Role of Competition-Induced Testosterone Dynamics. Neuroscience 286, 171–186. https://doi.org/10.1016/j.neuroscience.2014.11.029

Carter, C.S., 2007. Sex differences in oxytocin and vasopressin: Implications for autism spectrum disorders? Behav. Brain Res. 176, 170–186. https://doi.org/10.1016/j.bbr.2006.08.025

Cashdan, E., 1993. Attracting mates: Effects of paternal investment on mate attraction strategies. Ethol. Sociobiol. 14, 1–23. https://doi.org/10.1016/0162-3095(93)90014-9

Celec, P., 2013. Genetic Polymorphisms Related to Testosterone Metabolism in Intellectually Gifted Boys. PlosOne 8(1), e54751. https://doi.org/10.1371/journal.pone.0054751

Doherty, W.J., Erickson, M.F., LaRossa, R., 2006. An intervention to increase father involvement and skills with infants during the transition to parenthood. J. Fam. Psychol. 20, 438–447. https://doi.org/10.1037/0893-3200.20.3.438

Durante, K.M., Griskevicius, V., Simpson, J. a., Cantú, S.M., Li, N.P., 2012. Ovulation leads women to perceive sexy cads as good dads. J. Pers. Soc. Psychol. 103, 292–305. https://doi.org/10.1037/a0028498

Ebstein, R.P., Knafo, A., Mankuta, D., Chew, S.H., Lai, P.S., 2012. The contributions of oxytocin and vasopressin pathway genes to human behavior. Horm. Behav. 61, 359–379. https://doi.org/10.1016/j.yhbeh.2011.12.014

Edelstein, R.S., Anders, S.M. Van, Chopik, W.J., Goldey, K.L., Wardecker, B.M., 2014. Hormones and Behavior Dyadic associations between testosterone and relationship quality in couples. Horm. Behav. 65, 401–407.
Edelstein, R.S., Chopik, W.J., Kean, E.L., 2011. Sociosexuality moderates the association between testosterone and relationship status in men and women. Horm. Behav. 60, 248–255. https://doi.org/10.1016/j.yhbeh.2011.05.007
Eisenegger, C., Kumsta, R., Naef, M., Gromoll, J., Heinrichs, M., 2017. Testosterone and androgen receptor gene polymorphism are associated with confidence and competitiveness in men. Horm. Behav. 92, 93–102. https://doi.org/10.1016/j.yhbeh.2016.09.011
Ellison, P.T., 2003. Energetics and reproductive effort. Am. J. Hum. Biol. 15, 342–351. https://doi.org/10.1002/ajhb.10152
Fagan, J., Palkovitz, R., 2011. Coparenting and relationship quality effects on father engagement: Variations by residence, romance. J. Marriage Fam. 73, 637–653. https://doi.org/10.1111/j.1741-3737.2011.00834.x
Fisher, H.E., Aron, A., Brown, L.L., 2006. Romantic love: a mammalian brain system for mate choice. Philos Trans R Soc L. B Biol Sci 361, 2173–2186. https://doi.org/10.1098/rstb.2006.1938
Fleming, A.S., Corter, C., Stallings, J., Steiner, M., 2002. Testosterone and prolactin are associated with emotional responses to infant cries in new fathers. Horm. Behav. 42, 399–413. https://doi.org/10.1006/hbeh.2002.1840
Fleming, I.A., 1996. Reproductive strategies of Atlantic salmon: ecology and evolution. Rev. Fish Biol. Fish. 6, 379–416. https://doi.org/10.1007/BF00164323
Gangestad, S.W.W., Simpson, J. a. a, 2000. The evolution of human mating: trade-offs and strategic pluralism. Behav. Brain Sci. 23, 573–587; discussion 587-644. https://doi.org/10.1017/S0140525X0000337X
Gauthier, A.H., Smeeding, T.M., Furstenberg, F.F., 2011. Do We Invest Less Time in Children? Trends in Parental Time in Selected Industrialized Countries Since the 1960s. Ssrn. https://doi.org/10.2139/ssrn.1810844
Gavrilets, S., 2012. Human origins and the transition from promiscuity to pair-bonding. Proc. Natl. Acad. Sci. U. S. A. 109, 9923–8. https://doi.org/10.1073/pnas.1200717109
Gettler, L.T., McDade, T.W., Agustin, S.S., Feranil, A.B., Kuzawa, C.W., 2014. Testosterone, Immune Function, and Life History Transitions in Filipino Males (Homo sapiens). Int. J. Primatol. 35, 787–804. https://doi.org/10.1007/s10764-014-9749-5
Gettler, L.T., McDade, T.W., Agustin, S.S., Feranil, A.B., Kuzawa, C.W., 2013. Do testosterone declines during the transition to marriage and fatherhood relate to men's sexual behavior? Evidence from the Philippines. Horm. Behav. 64, 755–763. https://doi.org/10.1016/j.yhbeh.2013.08.019
Gettler, Lee T., McDade, T.W., Agustin, S.S., Kuzawa, C.W., 2011. Short-term changes in fathers' hormones during father-child play: Impacts of paternal attitudes and experience. Horm. Behav. 60, 599–606. https://doi.org/10.1016/j.yhbeh.2011.08.009
Gettler, L.T., Mcdade, T.W., Feranil, A.B., Kuzawa, C.W., 2012. Prolactin , Fatherhood , and Reproductive Behavior in Human Males 000, 1–9. https://doi.org/10.1002/ajpa.22058
Gettler, Lee T, McDade, T.W., Feranil, A.B., Kuzawa, C.W., 2011. Longitudinal evidence that fatherhood decreases testosterone in human males. Pnas 108, 16194–16199. https://doi.org/10.1073/pnas.1105403108

Gettler, L.T., Ryan, C.P., Eisenberg, D.T.A.A., Rzhetskaya, M., Hayes, M.G., Feranil, A.B., Bechayda, S.A., Kuzawa, C.W., Rzhet-, M., Hayes, M.G., Feranil, A.B., Bechayda, S.A., Kuzawa, C.W., Geoffrey, M., Alan, B., Agustin, S., 2017. The role of testosterone in coordinating male life history strategies: The moderating effects of the androgen receptor CAG repeat polymorphism. Horm. Behav. 87, 164–175. https://doi.org/10.1016/j.yhbeh.2016.10.012

Gonzalez-Bono, E., Salvador, A., Serrano, M.A., Ricarte, J., 1999. Testosterone, Cortisol, and Mood in a Sports Team Competition. Horm. Behav. 35, 55–62. https://doi.org/10.1006/hbeh.1998.1496

Gray, P.B., 2003. Marriage, Parenting, and Testosterone Variation Among Kenyan Swahili Men. Am. J. Phys. Anthropol. 122, 279–286. https://doi.org/10.1002/ajpa.10293

Gray, P.B., Campbell, B., 2009. Human male testosterone, pair bonding, and fatherhood. Endocrinol. Soc. Relationships 15, 119–131.

Jackson, J.J., Kirkpatrick, L.A., 2007. The structure and measurement of human mating strategies: toward a multidimensional model of sociosexuality. Evol. Hum. Behav. 28, 382–391. https://doi.org/10.1016/j.evolhumbehav.2007.04.005

James, J., Ellis, B.J., Schlomer, G.L., Garber, J., 2012. Sex-specific pathways to early puberty, sexual debut, and sexual risk taking: Tests of an integrated evolutionary–developmental model. Dev. Psychol. 48, 687–702. https://doi.org/10.1037/a0026427

Jasienska, G., Jasienski, M., Ellison, P.T., 2012. Testosterone levels correlate with the number of children in human males, but the direction of the relationship depends on paternal education. Evol. Hum. Behav. 33, 665–671. https://doi.org/10.1016/j.evolhumbehav.2012.05.001

Josephs, R.A., Sellers, J.G., Newman, M.L., 2006. The Mismatch Effect : When Testosterone and Status Are at Odds. J. Pers. Soc. Psychol. 90, 999–1013. https://doi.org/10.1037/0022-3514.90.6.999

Kaplan, H.S., Gangestad, S.W., 2004. Life History Theory and Evolutionary Psychology. Handb. Evol. Psychol. 2, 68–95. https://doi.org/10.1002/evan.1360020303

Kawasaki, T., Ushiyama, T., Ueyama, H., Inoue, K., Mori, K., Ohkubo, I., Hukuda, S., 1999. Polymorphic CAG repeats of the androgen receptor gene and rheumatoid arthritis. Ann. Rheum. Dis. 58, 500–502. https://doi.org/10.1136/ard.58.8.500

Kelsey, T W, Lucy Q. Li, Rod T. Mitchell, Ashley Whelan, Richard A. Anderson, W.H.B.W., 2014. A validated age-related normative model for male total testosterone shows increasing variance but no decline after age 40 years: Editorial comment. J. Urol. 9, 109346. https://doi.org/10.1016/j.juro.2015.02.062

Kruger, D.J., Fisher, M.L., 2005. Alternative male mating strategies are intuitive to women. Curr. Res. Soc. Psychol. 11, 39–50.

Kuo, Patty X., Ekjyot K. Saini, Elizabeth Thomason, Oliver C. Schiltheiss, R.G., 2016. Indivdidual Variation in Fathers' Testosterone Reactivity to Infant Distress Predicts Parenting Behaviours eith their 1-Year-Old Infants. Dev. Psychobiol. 58, 95–121. https://doi.org/10.1007/128

Manning, J.T., Bundred, P.E., Newton, D.J., Flanagan, B.F., 2003. The second to fourth digit ratio and variation in the androgen receptor gene. Evol. Hum. Behav. 24, 399–405. https://doi.org/10.1016/S1090-5138(03)00052-7

Marazziti, D., Canale, D., 2004. Hormonal changes when falling in love. Psychoneuroendocrinology 29, 931–936. https://doi.org/10.1016/j.psyneuen.2003.08.006

Marks, L., Palkovitz, R., 2004. American Fatherhood Types: The Good, the Bad, and the Uninterested. Father. A J. Theory, Res. Pract. about Men as Father. 2, 113–129. https://doi.org/10.3149/fth.0202.113

Mascaro, J.S., Hackett, P.D., Rilling, J.K., 2013. Testicular volume is inversely correlated with nurturing-related brain activity in human fathers. Proc. Natl. Acad. Sci. U. S. A. 110, 15746–51. https://doi.org/10.1073/pnas.1305579110

Mazur, A., Michalek, J., 1998. Marriage, Divorce and Male Testosterone. Soc. Forces 77, 315–330. https://doi.org/10.2307/3006019

McIntyre, M., Gangestad, S.W., Gray, P.B., Chapman, J.F., Burnham, T.C., O'Rourke, M.T., Thornhill, R., 2006. Romantic involvement often reduces men's testosterone levels--but not always: the moderating role of extrapair sexual interest. J. Pers. Soc. Psychol. 91, 642–651. https://doi.org/10.1037/0022-3514.91.4.642

Mckay, E.R., 2016. The Impact of Attachment, Parenting Attitudes, and Family History on Emotional, Behavioural, and Physiological Responses to Infant Crying. Dr. Diss. Texas Chri.

Miller, S.A., Gallie, D.R., Sleat, D.E., Watts, J.W., Turner, P.C., Wilson, T.M. a, 1988. A Simple Salting Out Procedure for Extracting DNA from Human Nucleated Cells. Nucleic Acids Res. 16, 883–893.

Muller, M.N., Marlowe, F.W., Bugumba, R., Ellison, P.T., 2009. Testosterone and paternal care in East African foragers and pastoralists. Proc. Biol. Sci. 276, 347–354. https://doi.org/10.1098/rspb.2008.1028

Muller, M.N., Wrangham, R.W., 2004. Dominance, aggression and testosterone in wild chimpanzees: A test of the "challenge hypothesis." Anim. Behav. 67, 113–123. https://doi.org/10.1016/j.anbehav.2003.03.013

Panizzon, M., Hsieh, T.-C., Carol, F., Hauger, R., Kremen, W., 2017. The Association Between Testosterone and Vitality: the Role of Genetic Variation in the Androgen Receptor. J. Urol. 197, e1223. https://doi.org/10.1016/j.juro.2017.02.2853

Perini, T., Ditzen, B., Hengartner, M., Ehlert, U., 2012. Sensation seeking in fathers: The impact on testosterone and paternal investment. Horm. Behav. 61, 191–195. https://doi.org/10.1016/j.yhbeh.2011.12.004

Puts, D.A., Pope, L.E., Hill, A.K., Cárdenas, R.A., Welling, L.L.M., Wheatley, J.R., Marc Breedlove, S., 2015. Fulfilling desire: Evidence for negative feedback between men's testosterone, sociosexual psychology, and sexual partner number. Horm. Behav. 70, 14–21. https://doi.org/10.1016/j.yhbeh.2015.01.006

Reynolds, A.C., Dorrian, J., Liu, P.Y., van Dongen, H.P.A., Wittert, G.A., Harmer, L.J., Banks, S., 2012. Impact of five nights of sleep restriction on glucose metabolism, leptin and testosterone in young adult men. PLoS One 7. https://doi.org/10.1371/journal.pone.0041218

Roney, J.R., Simmons, Z.L., Lukaszewski, A.W., 2010. Androgen receptor gene sequence and basal cortisol concentrations predict men's hormonal responses to potential mates. Proc. R. Soc. B Biol. Sci. 277, 57–63. https://doi.org/10.1098/rspb.2009.1538

Ryan, C.P., Georgiev, A. V., McDade, T.W., Gettler, L.T., Eisenberg, D.T.A., Rzhetskaya, M., Agustin, S.S., Hayes, M.G., Kuzawa, C.W., 2017. Androgen receptor polyglutamine repeat length (AR-CAGn) modulates the effect of testosterone on androgen-associated somatic traits in Filipino young adult men. Am. J. Phys. Anthropol. 163, 317–327. https://doi.org/10.1002/ajpa.23208

Sartor, O., Zheng, Q., Eastham, J.A., 1999. Androgen receptor gene CAG repeat length varies in a race-specific fashion in men without prostate cancer. Urology 53, 378–380.

Saxbe, D.E., Schetter, C.D., Simon, C.D., Adam, E.K., Shalowitz, M.U., 2017. High paternal testosterone may protect against postpartum depressive symptoms in fathers, but confer risk to mothers and children. Horm. Behav. 95, 103–112. https://doi.org/10.1016/j.yhbeh.2017.07.014

Sherman, G.D., Lerner, J.S., Josephs, R.A., Renshon, J., Gross, J.J., 2015. The Interaction of Testosterone and Cortisol Is Associated With Attained Status in Male Executives. J. Pers. Soc. Psychol. 109, 1–34. https://doi.org/10.1037/pspp0000063
Silverin, B., Wingfield, J.C., 1982. Patterns of breeding behaviour and plasma levels of hormones in a free-living population of Pied flycatchers, Ficedula hypoleuca. J. Zool. 198, 117–129. https://doi.org/10.1111/j.1469-7998.1982.tb02064.x
Simmons, Z.L., Roney, J.R., 2011. Variation in CAG repeat length of the androgen receptor gene predicts variables associated with intrasexual competitiveness in human males. Horm. Behav. 60, 306–312. https://doi.org/10.1016/j.yhbeh.2011.06.006
Soto-Gamboa, M., Villalón, M., Bozinovic, F., 2005. Social cues and hormone levels in male Octodon degus (Rodentia): A field test of the Challenge Hypothesis. Horm. Behav. 47, 311–318. https://doi.org/10.1016/j.yhbeh.2004.11.010
Spanier, G.B., 1976. Measuring Dyadic Adjustment: New Scales for Assessing the Quality of Marriage and Simialr Dyads. J. Marriage Fam.
Steiner, E.T., 2011. Testosterone and vasopressin in men ' s reproductive behavior. University of Nevada.
Stewart, S., Stinnett, H., Rosenfeld, L.B., 2000. Sex Differences in Desired Characteristics of Short-Term and Long-Term Relationship Partners. J. Soc. Pers. Relat. 17, 843–853. https://doi.org/10.1177/0265407500176008
Storey, A.E., Noseworthy, D.E., Delahunty, K.M., Halfyard, S.J., Mckay, D.W., 2011. The effects of social context on the hormonal and behavioral responsiveness of human fathers. Horm. Behav. 60, 353–361. https://doi.org/10.1016/j.yhbeh.2011.07.001
Storey, A.E., Walsh, C.J., Quiton, R.L., Wynne-Edwards, K.E., 2000. Hormonal correlates of paternal responsiveness in new and expectant fathers. Evol. Hum. Behav. 21, 79–95. https://doi.org/10.1016/S1090-5138(99)00042-2
Trumble, B.C., Cummings, D., von Rueden, C., O'Connor, K. a., Smith, E.A., Gurven, M., Kaplan, H., 2012. Physical competition increases testosterone among Amazonian forager-horticulturalists: a test of the "challenge hypothesis." Proc. R. Soc. B Biol. Sci. 279, 2907–2912. https://doi.org/10.1098/rspb.2012.0455
van Anders, S.M., Edelstein, R.S., Wade, R.M., Samples-Steele, C.R., 2012a. Descriptive experiences and sexual vs. Nurturant aspects of cuddling between adult romantic partners. Arch. Sex. Behav. 42, 553–560. https://doi.org/10.1007/s10508-012-0014-8
van Anders, S.M., Goldey, K.L., 2010. Testosterone and partnering are linked via relationship status for women and "relationship orientation" for men. Horm. Behav. 58, 820–826. https://doi.org/10.1016/j.yhbeh.2010.08.005
van Anders, S.M., Goldey, K.L., Kuo, P.X., 2011. The Steroid/Peptide Theory of Social Bonds: Integrating testosterone and peptide responses for classifying social behavioral contexts. Psychoneuroendocrinology 36, 1265–1275. https://doi.org/10.1016/j.psyneuen.2011.06.001
van Anders, S.M., Hamilton, L.D., Watson, N. V., 2007. Multiple partners are associated with higher testosterone in North American men and women. Horm. Behav. 51, 454–459. https://doi.org/10.1016/j.yhbeh.2007.01.002
van Anders, S.M., Tolman, R.M., Volling, B.L., 2012b. Baby cries and nurturance affect testosterone in men. Horm. Behav. 61, 31–36. https://doi.org/10.1016/j.yhbeh.2011.09.012
Walum, H., Westberg, L., Henningsson, S., Neiderhiser, J.M., Reiss, D., Igl, W., Ganiban, J.M., Spotts, E.L., Pedersen, N.L., Eriksson, E., Lichtenstein, P., 2008. Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. Proc. Natl. Acad. Sci. U. S. A. 105, 14153–14156. https://doi.org/10.1073/pnas.0803081105

Weisman, O., Zagoory-Sharon, O., Feldman, R., 2014. Oxytocin administration, salivary testosterone, and father–infant social behavior Omri. Prog. Neuropsychopharmacol. Biol. Psychiatry 49, 47–52. https://doi.org/10.1016/j.pnpbp.2013.11.006

Wilhelm, K., Parker, G., 1988. The Development of a Measure of Intimate Bonds. Psychol. Med. 18, 225–234.

Zilioli, S., Ponzi, D., Henry, A., Kubicki, K., Nickels, N., Wilson, M.C., Maestripieri, D., 2015. Interest in Babies Negatively Predicts Testosterone Responses to Sexual Visual Stimuli Among Heterosexual Young Men. Psychol. Sci. 1–5. https://doi.org/10.1177/0956797615615868

Zitzmann, M., Nieschlag, E., M., Z., 2003. The CAG repeat polymorphism within the androgen receptor gene and maleness. Int. J. Androl. 26, 76–83.

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

Chapter 2

Relationships between Peripheral Levels of Oxytocin and Vasopressin, Oxytocin (*OXTR*), *CD38*, and Vasopressin (*AVPR1A*) Receptor Genes, and Male Reproductive Strategies

by

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Abstract

Peripheral levels of oxytocin (OT) and vasopressin (AVP) have been suggested to reciprocally interact with a man's sociosexual environment to influence behavior. Similarly, variation in genotypes for the receptors of OT and AVP, as well as the glycoprotein CD38, have been associated with variability in partnering and parenting behaviors. To help understand the interactions between genetics, hormones, and behavior, fifty-two men (19-45 years) answered questionnaires and provided blood samples before and after romantic (two video clips, with partner) and paternal caregiving (RealCare baby doll) sessions. K-means clustering of demographic data, including health and family history, as well as relationship status, yielded three groups of males exhibiting behaviorally distinct reproductive strategies: Bachelor, Provider, Direct Father (3-Group Model). OT and AVP were quantified with enzyme immunoassays. Genotyping of receptor polymorphisms included three single nucleotide polymorphisms (SNPs) of *OXTR*, one SNP for *CD38*, and two microsatellites for *AVPR1A*. Reproductive strategies and genotypes were evaluated for their combined effects on OT and AVP levels. In general, men who had spent more time with children had higher OT than men who spent less time with children. However, there was genetic and endocrine evidence of alternative reproductive strategies in men, generally favoring the hypothesized 3-Group model for OT. Men in the Provider group had significantly higher OT than men in the Bachelor and Direct Father groups. Higher OT levels in men in the Provider group were specific to particular recessive homozygous genotypes: *OXTR* 2254298 (GG > AA/AG), *OXTR* 53576 (GG > AA/AG) and *CD38* (CC > AA/AC). Most interestingly, men in the Provider group experienced an increase in OT levels following the video session with their partner, but a decrease following the baby doll care-giving session. AVP levels did not differ significantly among men in the 3-Group model. However, an alternative model (RS-Partner/RS-Partner) indicated that men with low partner orientation and fewer copies of *AVPR1A*-rs1 had higher AVP levels than other men. These data suggest that many of the apparent

inconsistencies in male reproductive physiology and behavior are due to individual variation in both genotype and context, and further study is warranted.

Keywords: Oxytocin, CD38, Vasopressin, Reproductive strategies, Reproductive ecology, Social neuroendocrinology, Fathering, Oxytocin receptors, Vasopressin receptors.

Highlights:

- Endocrine and genetic evidence of alternative reproductive strategies in men
- 3-Group model better fit for Oxytocin (OT) levels than the RS-Partner/RS-Parent model
- OT levels higher for men in the Provider group who carried specific recessive genotypes for OT receptors
- Men in the Provider group experienced an increase in OT following partner interaction, but a decrease following caregiving
- Men with low partner orientation and fewer copies of *AVPR1A*-rs1 had higher AVP levels than men with high partner orientation and fewer copies of rs1

1. Introduction

Pair-bonding and parenting choices are heavily influenced by the underlying physiological processes, including peripheral hormones, neurotransmitters, and genetic characteristics. The interdependent relationships among the levels of physiological processing are illustrated by the finding that neurons involved in the release of oxytocin (OT) share interconnections with mesolimbic dopaminergic neurons (Melis & Argiolas, 2011), which serve both romantic and parental attachment through including sexual behavior and sexual preferences (Love, 2015). In addition, brain regions active in maternal attachment, are also activated by long-term romantic relationships (Acevedo et al., 2012). Given the evolutionary age of parent-offspring bonds, pair-bonds may have initially evolved from the neuroendocrine basis of maternal

nurturance (Fernandez-Duque et al., 2009) and then branched off as the foundation of adult pair bonds and paternal behavior in males. The connection between brain function, sexual behaviors, and parenting behaviors suggests that research should focus on both the relationship between a man and his infant, as well as, a man and his partner.

Correlational studies have assessed the relationship between oxytocin (OT), arginine vasopressin (AVP), and human social behaviors. Most of these studies utilized peripheral measures of the hormones, despite the long-standing debate about whether peripheral levels of OT and AVP are representative of central levels as they can not cross the blood brain barrier (Carson et al., 2015, 2014; McCullough et al., 2013; Valstad et al., 2017). Administration of intranasal AVP also resulted in elevated blood levels of AVP (Landgraf and Neumann, 2004), but there is evidence that baseline plasma levels do not reflect central levels. Although Ebstein et al. (2012, see also Carson, 2014, 2015) reviewed current literature examining OT and AVP pathway genes and human behavior and concluded that plasma OT measurements are certainly related to central activity, other work suggests that there is no relationship for baseline levels (i.e., without intranasal application, Kagerbauer et al., 2013; McCullough et al., 2013), including a recent meta-analysis (Valstad et al., 2017). That peripheral levels are reflective of central activity is important to the study of human behavior as it allows for non-invasive measurement of hormone levels (for example, see Feldman et al., 2013).

1.1. Peripheral Oxytocin & Vasopressin

OT has been found to play a role in social relationships, in part due to its anxiolytic and anti-stress effects which are important for bonding (Neumann, 2008). Some effects are tied closely to the social context, such as the bias to favor in-group members over out-group members (Luo et al., 2015). The significance of the romantic relationship was shown as intranasal administration of OT which resulted in monogamously-mated men not allowing an attractive female to closely approach them whereas approach distances for non-monogamous men were not

affected (Scheele et al., 2012). One interesting possibility that has not yet been explored is that OT responses to a relationship partner transfer to OT responses involving progeny, a possibility that will be examined in the current study.

It is important to consider the effect that early childhood experience has on the development of social behaviors and their biological foundations. Fries et al. (2005) showed that children who experienced early childhood neglect had lower overall levels of OT and AVP than family-reared children. Additionally, OT levels increased in family-reared children following physical interaction with their mothers, whereas neglected children did not experience such a change. Based on these results, one would expect sensitive parenting to yield a well-developed OT/AVP system. In a study of fathers' interactions with their infants, peripheral levels of OT in the father were positively correlated with affect synchrony between father and infant (Feldman et al., 2010). Furthermore, higher levels of paternal sensitivity were associated with more infant-father attachment security (Lucassen et al., 2011). These studies suggest that failure to receive species-typical care disrupts the normal development of the OT and AVP systems whereas sensitive parenting by both parents supports functionally dynamic OT and AVP systems, highlighting the importance of creating a supportive environment for both parents to provide childcare.

In turn, the establishment of secure adult attachment is important as it has been shown that adult attachment predicts maternal brain and OT response to infant cues (Strathearn et al., 2011), which then allows sensitive parenting. Not only is responsive and caring parental care essential to the development of the infant, but also to the development of that infant's ability to form secure attachments in adulthood, including pair-bonding and paternal bonding (Atzil et al., 2011; Sroufe, 2005).

One factor inherent in family dynamics is the physical interaction between family members. The foundation of maternal attachment in rats is the frequency of licking and grooming

behaviors, rather than simply the genetic disposition of the mother (Champagne, 2008). This early experience plays a role in the establishment of OT receptors and thus affects the subsequent maternal behavior of these young when they mature. Consistent with these data, adult women who were abused during childhood have lower OT levels than controls (Heim et al., 2009). Higher endogenous OT levels were associated with greater affect synchrony between parents and infants, as well as longer play sessions (Feldman et al., 2010). Taken together, these data, and the results of genetic studies (e.g., Feldman et al., 2012), show the importance of the interaction between both genes and environment (i.e., epigenetic effects) on the OT system of humans.

1.2. Genotype Variation in OXTR, CD38, and AVPR1A

How genes associated with OT and AVP affect behavior has been increasingly studied in the past 20 years. This research has led to discoveries about genomic characteristics, tissue expression, chromosomal localization, and regional mapping of the receptor genes (Thibonnier et al., 1996). The *OXTR* gene is present as a single copy on each of the two chromosomes in the human genome mapped to the locus 3p25-3p26.2. Deletion experiments show that approximately 1000bp upstream of the coding region is needed for expression of *OXTR* (Inoue et al., 1994). Variants (SNPs) of the *OXTR* gene have been examined relative to behavioral phenotypes, such as parenting (Feldman et al., 2012; Walum et al., 2012) and pair-bonding (Schneiderman et al., 2014), as well as psychological disorders like Autism Spectrum Disorder (ASD, Campbell et al., 2011). Specifically, alleles of the *OXTR* SNPs rs7632287, rs2254298, and rs53576 are of particular interest in parental and partner behaviors (personal communication, Dr. Hasse Walum), possibly in conjunction with other hormone systems. For example, an associative study of fetal testosterone (T) exposure indicated that men with the GG genotypic variation of *OXTR* rs53576 and low fetal T displayed more empathy than men with GG alleles and high fetal T (Weisman et al., 2015).

Studies have also indicated that the multifunctional transmembrane glycoprotein, ADP-ribosyl cyclase (*CD38)*, is highly expressed has been associated with peripheral OT levels. For example, *CD38* knockout (KO) mice showed a reduction of OT in both CSF and plasma OT (Jin et al., 2007), even though hypothalamic and pituitary stores of OT in secretory vesicles were elevated. These results suggest that OT release was selectively impaired in the *CD38* mutants. Interestingly, maternal nurturing and social memory deficits in *CD38* mutant mice could be restored by replacing OT via peripheral injection or direct injection into the third ventricle (Bartz and McInnes, 2007). Studies in humans have shown significant differences in behavior and physiology by *CD38* genotype, such as reduced OT levels and decreased parental gaze and touch (CC genotype; Feldman et al., 2012).

There are three known receptors for AVP, including *AVPR1A*, *AVPR1B*, and *AVPR2*, all of which, with the exception of *AVPR2*, are expressed in the brain (Caldwell et al., 2008). Reproductive behaviors, age of first sexual experience and number of sexual partners have been associated with variations in gene alleles for *AVPR1A* (AGAT genotype and (TG)x(TC)y polymorphism) and *OXTR* (CA; Prichard et al., 2007). Specific AVP alleles, such as the complex (CT)4-TT-(CT)8- (GT) 24 repeat (rs3) and a (GATA)14 tetranucleotide repeat (rs1), have been found to relate to decreased partner bonding, marital problems, and, most significantly, perceived marital quality as described by the spouse (Walum et al., 2008). Additionally, a study of *AVPR1A* in sibling pairs suggest that *AVPR1A* mediates social behavior in humans and that a specific genetic element is linked to perceived sibling relationships (Ebstein et al., 2012). It is likely that contextual cues determine the extent to which gene variation influences the expression of hormones and, therefore, related sexual and reproductive behaviors (i.e., Wallen, 2001).

1.3. Reproductive Strategies

Maximizing reproductive success requires prioritization of resources resulting in a variety of trade-offs in physiology and behavior. Researchers have proposed that pair-bonding, and by extension paternal care, evolved as an alternative strategy for males to compete with more dominant men (Gavrilets, 2012). Research in this area has focused on men investing in either long-term partnering and parenting ('dads') or short-term mating opportunities ('cads') as resources and time limit engagement in both strategies simultaneously (Cashdan, 1993). Other researchers have considered as many as four categories of men: the "new, involved father"; the "good provider"; the "deadbeat dad"; and the "paternity-free man" (Marks & Palkovitz, 2004). To facilitate the evaluation of male reproductive strategies and their underlying physiological correlates, I have proposed a comprehensive model of male reproductive strategies as they relate to the OT/AVP system (3-Group Model) and compared statistical analyses of this model to an alternative model (RS-Partner/RS-Parent Model). The 3-Group Model divides men into three categories based on measures of both partnering and parenting variables: Bachelor, Provider, and Direct Father. The RS-Partnering/RS-Parenting Model separates partnering and parenting variables to create distinct, but interrelated, categories of individuals (see Chapter 1 for details).

1.4. Objectives

The first study will experimentally examine hormone levels and variation in receptor genes as they relate to both pair-bonding and parenting behaviors in the context of human male reproductive strategies. The 3-Group and the RS-Partner/RS-Parent models will be assessed in relation to peripheral levels of OT and AVP as well as in interactions between hormone levels and (a) responses to pair-bonding and parenting situations and (b) variation in hormone receptor genotypes. I expect OT levels to be higher for men carrying genotypes previously associated with nurturant and/or partner-oriented behavior (*OXTR* 2254298, AA/AG > GG; *OXTR* 1042778, GG/GT > TT; *OXTR* 53576, GG > AA/AG; CD38, AA/AC > CC; Bakermans-Kranenburg and

van Ijzendoorn, 2008; Feldman et al., 2013, 2012; Hostinar et al., 2014; Prichard et al., 2007; Walum et al., 2008, 2012). Similarly, I expect men carrying fewer base pairs for AVPR1A-rs1 (Tansey et al., 2011) and/or fewer base pairs (possibly 334 bp specifically) for *AVPR1A*-rs3 (Nishitani et al., 2017; Walum et al., 2008) to display less responsive partner and parenting behaviors, as well as higher AVP levels.

2. **Methods**

2.1. Study population

A total of 52 men were recruited from prenatal, breastfeeding, and infant care classes at the Women's Health Centre located in the Health Sciences Centre, St. John's, Newfoundland and Labrador. Participants were also recruited through informational talks and interdepartmental newsletters at Memorial University of Newfoundland, as well as through community flyers, posters, and blood donor clinics. Institutional approval from Human Research Ethics Authority (Memorial University of Newfoundland – 13.105) was obtained prior to data collection for this study on August 22, 2013. There were a total 44 men in the partner video session and 46 men in the baby doll session. A total of 38 men participated in both the video and the doll session. All men were English speaking and between the ages of 19 and 45 years old ($M = 31.24 \pm 7.313$). Participant ages were selected to avoid the hormonal peaks following the end of puberty and the declines of aging males (Kelsey et al., 2014). Participants self-reported their place of origin as Newfoundland (n=33), other parts of Canada (n=11), or other (n=8). Participants reported their parental status as having a baby (n=11), having older children (n=12), or no children (n=29). Of interest, of the men that reported not having children, 17 reported wanting children in the future, while 12 men reported not wanting children.

2.2. Procedure

Experimental sessions occurred in the Psychology Department of Memorial University from December 2013 to December 2015 between 1200-1600 hours, with subsequent sessions

occurring approximately one week later. Men participated in 1-2 experimental sessions and were asked to refrain from caffeine, alcohol, and sexual interaction for the 24-hours prior to their session. Participants were also asked to refrain from intense exercise just prior to the session. For all sessions, participants filled out questionnaires related to personal demographics, relationship dynamics, interactions with children, and their experiences during the experimental sessions. Once the questionnaires were complete, I took a venous blood sample for the analyses of OT and AVP. Blood samples were also sequenced for receptor polymorphisms of *OXTR*, *CD38*, and *AVPR1A*. Following blood samples, there were two experimental interaction sessions: the baby doll interaction and the romantic videos. The baby doll session utilized the RealCare baby doll (see van Anders, Tolman, & Volling, 2012) as an infant proxy. The video session included the man and his partner (if applicable) watching two romantic video clips (see Steiner, 2011). Approximately 30-mins after the first blood sample, participants gave a second blood sample and reported their experiences during the session.

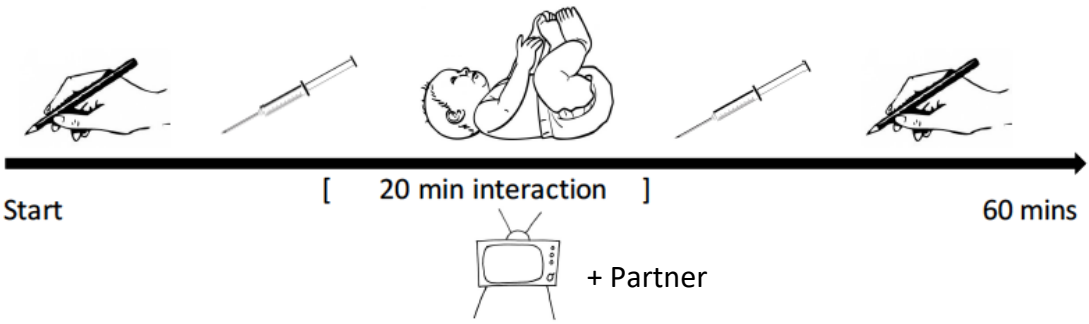


Figure 1. Experimental method for romantic video session and RealCare baby doll session.
* please note that the second blood sample was approximately 30-mins after the first blood sample

2.3. Questionnaire data

Questionnaires included demographic information, the Dyadic Adjustment Scale (DAS-Spanier, 1976), the Intimate Bond Measure (IBS –Wilhelm & Parker, 1988), and the post-interaction questionnaire (see Appendices). The demographic information questionnaire covered topics such as age, weight, smoking habits, profession, place of origin, relationship status, parenting experience, and sexual relations and history. Variables, such as "Time with Kids", were interpreted from the raw demographic data. For example, "Time with Kids" was categorized by high ($\geq$10 hours per week) or low (<10 hours per week) interaction (defined as being primarily responsible for child's care) with children.

The DAS included thirty-two scaled questions (i.e., 0 =Always Disagree, 5= Always Agree; "Which of the following statements best describes…"; "yes" or "no" questions) related to interaction quality across relationship topics. Scoring for the DAS is broken down into four categories: perception, affection, consensus, and cohesion, as well as the total score (described in detail in Spanier, 1976). Although the scoring method for the DAS includes these five interdependent subcategories, we decided to use only the total DAS score as a representation of relationship quality for our statistical analyses (as per Walum et al., 2008).

The IBS included 24 questions regarding partner interaction quality (Wilhelm and Parker, 1988). Twelve of the questions relate to care behaviors and twelve of the questions relate to control behaviors. For example, participants responded to the statement "my partner wants to know where I am at all times". Answers were rated as "true", "moderately", "somewhat", or "not at all", with higher numbers representing higher levels of care and/or control in the relationship.

2.4. Pair-bonding Interaction: Video session

During the video session participants watched two romantic videos (one sexually romantic and one emotionally romantic) with their partner (if applicable). The sexually romantic video was an excerpt from *The Notebook* (2004 -PG-13), depicting two estranged lovers reuniting

in a mildly sexual scene. The emotionally romantic video was a short film titled *Signs* (2008), depicting an office romance at a distance with the use of "signs" (sheets of paper with short notes on them). All session were approximately 60-minutes long, with approximately 30-minutes between blood samples, and occurred in the same laboratory setting. The only hormonal difference between men who were in a relationship and those not was that the men in relationships had lower baseline cortisol in the video session than non-partnered men ($t_{40} = 2.2$, $P = 0.04$).

2.5. Parenting Interaction: RealCare baby doll session

The RealCare baby (manufactured by RealityWorks) approximated a 6-month old male infant (as used in van Anders et al., 2012). It was dressed in a blue shirt and footed pants. Once activated, the doll behaved as directed by the same selected care program for each participant. During the 20-minute interaction the doll 'requested' four care events: bottle feed, burp, diaper change, and gentle rocking. Each man was responsible for identifying and providing the care required. The doll recorded successful vs. unsuccessful care attempts (percentage out of 100), number of minutes spent crying, and any instances of rough handling (deducted points from total). Data from the doll was downloaded upon completion of the interaction and participants were asked to fill-out a follow-up questionnaire about their experiences during the session. The doll has not been formally validated but the change in OT in a small sample of new fathers tested with their own babies in this study was marginally correlated with their OT change with the baby doll ($r = 0.84$, $p = .08$, $n = 5$).

2.6. Sample collection and processing protocol

Blood samples were collected by standard protocol for venous blood collection into 8ml EDTA filled plastic tubes. Samples were kept on ice for up to one hour and then centrifuged at 1,6000g for 15-mins. Once centrifuged, plasma was aliquoted into a 2-ml O-ring tubes and stored at -20°C for 1-3 months for enzyme immunoassay of OT and AVP. The remaining sample (white

and red blood cells) was stored at 4°C for up to 1-week for DNA extraction in batches by salting out method (Miller et al., 1988). Extracted DNA samples were frozen at 20°C for genotyping.

2.7. OT assay

OT enzyme immunoassays (pg/mL) were performed at the Pollak Laboratory at McGill University, Montreal, Canada. Plasma samples were processed with an Enzo Life Sciences assay kit (Oxytocin ELISA kit Catalog No. ADI-900-153), following standard protocol as described in the kit insert. Samples were run in duplicate, with the average of the two used for the final data point; intra-assay coefficient of variation values were calculated. The intra-assay values were an average of 2.84% for all samples. Samples were assayed without extraction as discussed in Carter et al. (2007) and Ebstein et al. (2012). Additionally, comparisons in this study focused on comparisons of individuals over time and with the same context so presumably the relative differences would be the same.

2.8. AVP assay

AVP enzymeimmunoassays (pg/mL) were also performed at the Pollak Laboratory at McGill University, Montreal, Canada. Plasma samples were processed with an Enzo Life Sciences assay kit (arg8-Vasopressin EIA kit Catalog No. ADI-900-017), following standard protocol as described in the kit insert. Samples were run in duplicate, with the average of the two used for the final data point, and intra-assay coefficient of variation values were calculated. The intra-assay coefficient of variation values were an average of 3.52% for all samples.

2.9. Oxytocin receptor (OXTR) SNPs and CD38

Three Single Nucleotide Polymorphisms (SNPs, *OXTR* rs53576, rs2254928, rs1042778) associated with the oxytocin receptor gene and one SNP associated with CD38 were amplified using Polymerase Chain Reactions (PCRs). SNP amplification products were cleaned, prepared for, and sequenced on a 48-capillary sequencer (3730 DNA Analyzer, Applied Biosystems Inc.,

USA). PCRs contained 12.5μL 2X Qiagen type-it master mix, 1μL 10μM F primer, 1μL 10μM R primer, 1μL gDNA (~25 ng), and 9.5 μL dH_2O.

Table 1. Primers used to genotype the *OXTR* and CD38 SNPs

OXTR rs2254298	F: 5'-CCCAGAGGTCTGTGGGTGTA-3'
	R: 5'-GTCAGGGAGGAGCTGTTCTG-3'
OXTR rs1042778	F: 5'-TGGGTTCAGGGTGGTAGAAG-3'
	R: 5'-AGGCTGTGCTGGCATAAGTG-3'
OXTR rs53576	F: 5'-GCCCACCATGCTCTCCACATC-3'
	R: 5'-GCTGGACTCAGGAGGAATAGGGAC-3'
	R: 5'-GCTGGACTCAGGAGGAATAGGGAC-3'
CD38 rs3796863	F: 5'-GGTGCACAGACCACTTAGCA-3'
	R: 5'-TCGGAAGAGAGGAAAGCAA-3'

PCR conditions consisted of an initial denaturation at 95°C for 5 minute, 35 cycles at 95°C for 30 seconds, 64°C for 45 seconds, and 72°C for 1 minute, with a final elongation at 72°C for 5 minute. PCR conditions varied for rs2254298 by omitting the "72°C for 1 minute" step. PCR products were purified for cycle sequencing using Pall AcroPrep 96 Multi-Well filter plates (Pall Life Sciences, Port Washington, USA) according to manufacturer's instructions. Sequencing was carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc.) following the manufacturer's protocol, and purified via ethanol precipitation. Sequencing products were electrophoresed in an Applied Biosystems 3730 DNA Analyzer using Sequencing Analysis v. 5.2 Software. Sequences were edited and aligned using Sequencher v4.8 (Gene Codes, Ann Arbour, USA). Single nucleotide polymorphisms (SNPs) were identified by comparison of

individual consensus contigs (overlapping gel sequences) and confirmed by visual inspection of sequence chromatograms. Heterozygous sites within individuals were identified by double peaks of approximately equal intensity (usually lower than single peaks at nearby sites), in both the forward and reverse read.

2.10. AVP Microsatellite Analyses

Extracted DNA was screened with rs1 and rs3 in a single multiplex reaction. All reactions contained (in addition to primers) 12.5μL 2X Qiagen type-it master mix, 1μL 10μM F primer, 1μL 10μM R primer, 1μL gDNA (~25 ng), and 9.5 μL dH_2O.

Table 2. Primers used to genotype the AVPr1A Microsatellites RS1 and RS3.

AVPR1A-rs1	F: 5'-AGGGACTGGTTCTACAATCTGC-3'
	R: 5'-ACCTCTCAAGTTATGTTGGTGG-3'
AVPR1A-rs3	F: 5'-TCCTGTAGAGATGTAAGTGC-3'
	R: 5'-GTTTCTTTCTGGAAGAGACTTAGATGG-3'

All PCRs included a no template control. Thermal cycling was performed in a GeneAmp 9700 Thermal Cycler (Applied Biosystems Inc., Foster City, California, USA). Multiplex I, II and Ma18 were amplified with the following conditions: 95˚C for 5 minutes, followed by 30 cycles of 95˚C of 30 seconds, 55˚C for 30 seconds and 72˚C for 40 seconds, and a final extension of 72˚C for 10 minutes. Microsatellite PCR product were diluted and run with an internal standard (LIZ500; Applied Biosystems Inc.) on an Applied Biosystems 3730 DNA Analyzer using GeneScan software (Applied Biosystems Inc.), and analyzed using Peak Scanner Software v1.0. Number of alleles from the forward and reverse run were averaged for analyses.

2.11. Statistical analyses

All statistical analyses were performed in SPSS version 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Results were considered significant at $p<0.05$. Data exploration was carried out following the protocol described in Zuur et al. (2010). Questionnaires and demographic data were utilized to calculate reproductive strategies by k-means clustering (MacQueen, 1967) of partnering and parenting behaviors from questionnaires for the 3-Group model (Bachelor, Provider and Direct Father) and the alternative concept of the RS-Partner/RS-Parent model (high or low partner or parent orientation). One-way ANOVAs were used to test differences between the 3-Groups, with Bonferroni correction for multiple tests. t-tests were used for the two group analyses: RS-Partner/RS-Parent. In the case of nominal data, Fisher's test was utilized.

Genotype data for OT were dichotomized based on previous literature identifying the dominant alleles for that particular receptor. Specifically, the individuals were classified into one of two genotype groups for each receptor based on previous research predicting associations between genotypes and socioreproductive behaviors (Table 3).

Models were evaluated for OT/AVP levels by repeated measures GLM with the two or three hypothesized reproductive groups, dichotomized genotype data and the video/baby doll sessions (repeated measures) as the independent variables, and baseline and reactive OT levels as the dependent variables.

Table 3. Associations between oxytocin genotypes and parenting behaviors.

Author(s)	Genotype	Behavior
Feldman, 2012		
OXTR rs1042778	GG/GT > TT	Parental Touch
CD38 rs3796863	AA/AC > CC	Parental Touch
Marsh et al., 2012		
OXTR rs53576	GG > AA/AG	Infant Facial Preference
Bakermans-Kranenburg & van Ijzendoorm, 2008		
OXTR rs53576	GG > AA/AG	Sensitive Parenting
Chen et al., 2011		
OXTR rs2254298	AA/AG > GG	Secure Attachment

AVP data was dichotomized based on previous literature that indicated length of the microsatellite being the determining factor in behavioral and physiological differences (Tansey et al., 2011; Walum et al., 2008). The rs1 variant was dichotomized by the median/mode (1 > 318, 2 ≤ 318), while the RS3 variant was dichotomized by the mean/median/mode (1 > 345, 2 ≤ 345).

Demographic data were analyzed with t-test or ANOVA. The 3-Group and RS-Partner/RS-Parent models were analyzed with Repeated Measures GLMs with OT/AVP as the dependent variable and the specific reproductive model, session, and four SNPs/Micro as the independent variables.

3. Results

3.1. Demographics

One-way ANOVAs and t-tests were used to evaluate demographic data with baseline and reactive hormone levels. Men's baseline and reactive levels of OT and AVP levels did not differ significantly during the video session or the baby doll session (all *ps* > 0.17, Power .05 - .27).

Likewise, there were no significant differences in OT or AVP between individuals for age, place of origin, relationship status, or desire to have children. Two variables, 'fatherhood status' (father/non-father) and time with children (more or less than 10 hr per week) were associated with OT levels. OT levels showed stability across tests (range of r, .42 - .60, $P < 0.02$). The variable 'time with children' overlapped extensively with fatherhood status except that one father reported spending little time and one non-father reported spending a lot of time with children. Fathers had higher OT than non-fathers only in their reactive levels after the video session (fathers, 970.9 ± 90.4 pg/mL; non-fathers, 800 ±31.1 pg/mL; $t_{41} = 2.09$, $p = 0.043$). Differences were more pronounced for 'time with children': compared to men reporting little time with children, men who reported spending more time with children had higher baseline ($F_{1,42} = 5.28$, $p = 0.02$, $eta^2 = .11$, Power = .62) and reactive OT levels ($F_{1,41} = 7.32$, $p = 0.01$, $eta^2 = .13$, Power = .69; Figure 2a) in the video session, and higher reactive OT levels than men who spent less time with children in the baby doll session ($F_{1,44} = 4.46$, $p = 0.04$, $eta^2 = .09$, Power = .51; Figure 2b). There were no significant differences for 'time with children' in OT levels at baseline in the baby doll session ($eta^2 = .08$, Power = .43) and no significant differences in AVP associated with any demographic factor. OT and AVP levels were correlated within and across sessions (range of r, OT: 0.42-.0.60, $P < 0.001$, n = 37, AVP: 0.73-0.84, Ps < 0.04, n = 37).

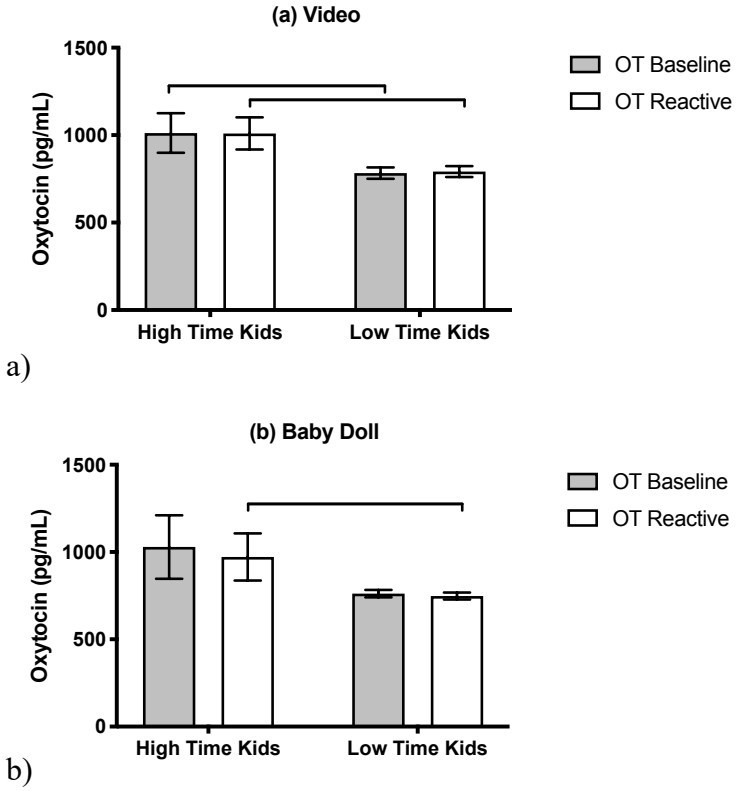


Figure 2. OT levels (pg/mL; M±SE) were higher before (video session) and after (both sessions) for men reporting that they spent more (N = 16) compared to less (N= 28) time with children: (a) Video session; (b) Baby Doll session.

The first two of the following four analyses examined how hormone levels (OT or AVP) in men in the three reproductive groups are affected by their receptor genotypes and their responses in the video and baby doll tests. The second two analyses evaluated the same variables in the RS parent/RS partner model. Models were evaluated for OT/AVP levels by repeated measures GLM with the two or three hypothesized reproductive groups, dichotomized genotype data and the video/baby doll sessions (repeated measures) as the independent variables, and baseline and reactive OT levels as the dependent variables.

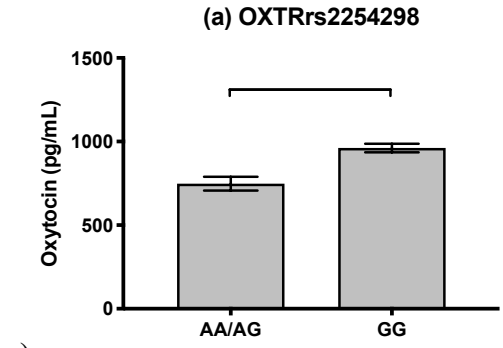
3.1.1. 3-Group model for OT

Men in the three groups differed significantly in their OT levels ($F_{2,18} = 5.87$, $p = 0.01$). Pairwise comparisons of OT levels indicated that men in the Provider group had significantly higher OT than men in the Direct Father ($p < 0.01$) or Bachelor groups ($p < 0.01$). Additionally, within-subject tests indicated that were significant interactions between group membership and the hormonal response to the two sessions ($F_{2,18} = 7.17$, $p < 0.01$ $eta^2 = .44$, Power = .89) as well as to before and after the session ($F_{2,18} = 3.81$, $p = 0.04$, $eta^2 = .30$, Power = .62) sessions. Specifically, men in the Provider group had higher OT levels in the baby doll session than in the video session and their levels decreased overall (both sessions) more than the other two groups.

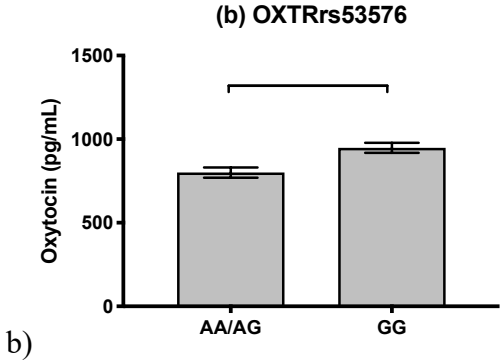
Men with homozygous recessive alleles had higher OT levels than other men for the three SNPs that yielded significant differences. Men with the GG genotype of *OXTR* 2254298 had higher OT levels than men with the AA and AG genotypes ($F_{1,18} = 31.77$, $p < 0.01$, $eta^2 = .64$, Power = 1.0; Figure 3a). Men with the GG genotype of *OXTR* 53576 had higher OT than men with AA and AG genotypes ($F_{1,18} = 17.26$, $p < 0.01$, $eta^2 = .49$, Power = .98; Figure 3b). Men with the CC genotype of *CD38* had higher OT levels than men with AA and AC genotypes ($F_{1,18} = 11.65$, $p < 0.01$, $eta^2 = .39$, Power = .90; Figure 3c). There were no significant differences between men with the GG and GT or TT genotype of *OXTR* 1042778.

There were significant interactions between the three groups and specific genotypes for *OXTR* 2254298 ($F_{2,18} = 40.19$, $p < 0.01$, $eta^2 = .82$, Power = 1.0), *OXTR* 53576 ($F_{2,18} = 102.73$, $p < 0.01$, $eta^2 = .85$, Power = 1.0), and *CD38* ($F_{2,18} = 76.27$, $p < 0.01$, $eta^2 = .89$, Power = 1.0; Figure 4), but not for *OXTR* 1042778. In all SNPs, the homozygous recessive genotype in the Provider group had the higher OT levels than men with that genotype in the Bachelor and Direct father groups and higher OT levels than the other men in the Provider group. For *OXTR* 2254298, men with the GG genotype in the Provider group had higher OT than men with AA and AG ($t_{11} = 7.17$, $p < 0.001$) and men with GG in the Bachelor or Direct Father group ($F_{2,38} = 37.45$, $p < 0.001$). For

OXTR 53576, men with the GG genotype in the Provider group had higher OT than men with AA and AG alleles ($t_{11} = 9.21$, $p < 0.001$) and men with GG in the Bachelor or Direct Father group ($F_{2,24} = 65.63$, $p < 0.001$). For *CD38*, men with the CC genotype in the Provider group had higher OT than men with AA and AC ($t_{11} = 9.20$, $p < 0.001$) and men with CC in the Bachelor or Direct Father group ($F_{2,23} = 50.67$, $p < 0.001$).



a)



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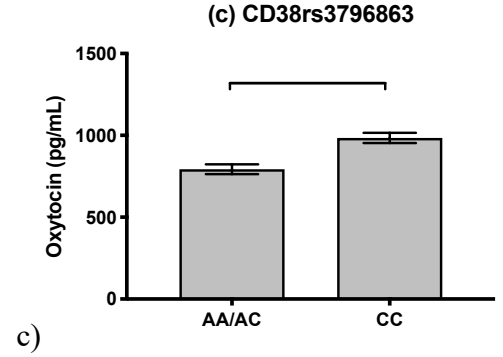
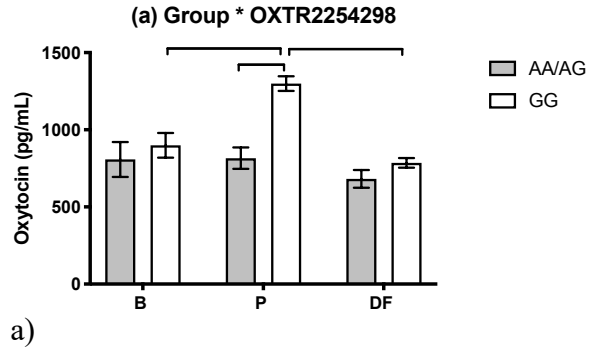
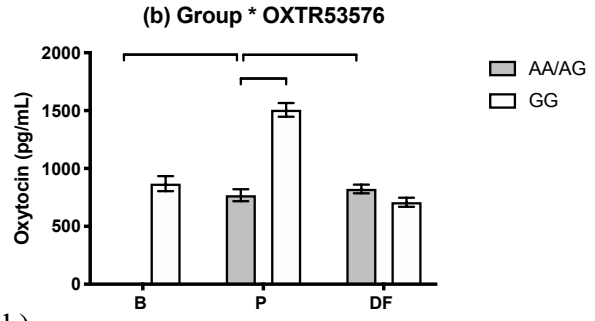


c)

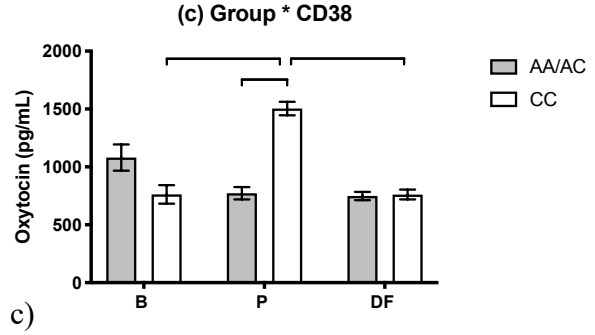
Figure 3. OT levels (pg/mL; M±SE) were higher for men with the homozygous recessive genotype in a) *OXTR* 2254298 (AA/AG=9, GG=28), b) *OXTR* 53576 (GG=17, AA/AG=20), and c) *CD38* (AA/AC=18, CC=19). There was no significant relationship between genotype and OT level for *OXTR* 1042778. Bars indicate significant difference.



a)



b)



c)

Figure 4. OT levels (pg/mL; M±SE) by group membership and genotype (Bachelor = 3, Provider = 10, Direct Father = 24). (a) *OXTR* 2254298 men with the GG genotype in the Provider group had higher OT than men with AA/AG and men with GG in the Bachelor or Direct Father group; (b) *OXTR* 53576 men with the GG genotype in the Provider group had higher OT than men with AA/AG and men with GG in the Bachelor or Direct Father group; (c) *CD38*-rs3796863 men with the CC genotype in the Provider group had higher OT than men with AA/AC and men with CC in the Bachelor or Direct Father group. Bars indicates significant differences.

3.1.2 3-Group model for AVP

There were no significant relationships between AVP levels and the 3-Group model.

3.2.1 RS-Partner/RS-Parent model for OT

There were no significant relationships between OT levels and the RS-Partner/RS-Parent model in these analyses.

3.2.2 RS-Partner/RS-Parent model for AVP

There were no significant main effects in the model, however there was a significant interaction between RS-Partner and *AVPR1A*-rs1 ($F_{1,21} = 5.85$, $p = 0.03$; Fig. 5). Men with low partner orientation and fewer copies of rs1 had higher AVP levels than men with high partner orientation and fewer copies of rs1 ($t_{25} = 2.41$, $p = 0.02$). However, there were no other significant differences in partner orientation and *AVPR1A*-rs1.

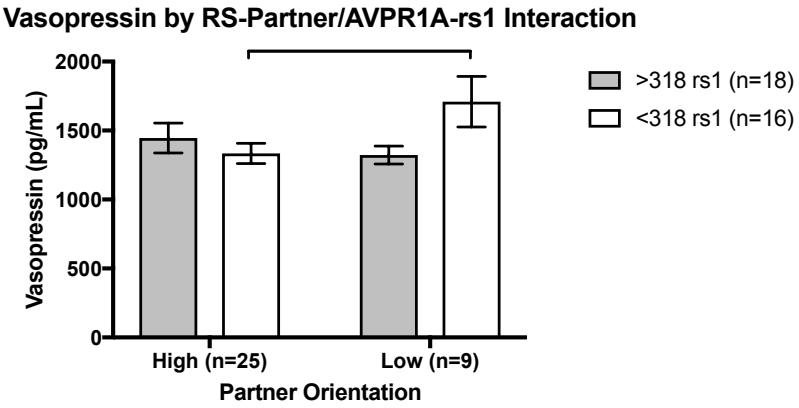


Figure 5. AVP levels (pg/mL; M±SE) were higher in men with low partner orientation and fewer copies of AVPR1A-rs1than men with high partner orientation and fewer number of *AVPR1A*-rs1 .

4. Discussion

4.1. Demographics

Compared to men with little child exposure, men who spent a lot of time with children had higher OT levels, both before and after the video as well as after the baby doll session. Although fatherhood status was also linked to higher OT levels, it appeared that time with children was a more important factor. A similar, but opposite, relationship was shown for testosterone, with men showing decreases when they became fathers, but these decreases were most pronounced in men who engaged in more child care (Gettler et al., 2011).

4.2. 3-Group model

Our results supported individual differences in men's reproductive strategies in relation to OT levels. Despite our prediction of higher OT levels in men in the Direct Father group, it was men in the Provider group who consistently displayed the highest OT levels. I suggest that anticipation of the experimental session may have initiated hormone changes in men in the Provider group prior to arrival at the session as their reproductive role may be defined by an ability to adapt to their current reproductive context. As previous research has indicated, increased OT levels have been associated with participation in a positive romantic relationship (Grewen et al., 2005; Marazziti and Canale, 2004), as well as being a father (Gordon et al., 2010; Mascaro et al., 2014). A difficulty in interpretation may arise here since a high proportion of men in the Provider group were fathers (62%, compared to 46% in the Direct Father group). However, since fatherhood status appears to play only a minor role in variation in our results and fathers and non-fathers did not differ in OT levels within the Provider group, it seems reasonable to interpret the results in terms specific reproductive group membership.

The results of the current analysis of OT receptor alleles generally found higher OT levels in individuals with recessive homozygous genotypes, results that contrast with those of the previous major study on parental behavior (Feldman et al., 2012). Men with the recessive GG

genotype of *OXTR* rs2254298, particularly those in the Provider group, had higher OT levels than men with AA or AG genotypes. In contrast, Feldman et al. (2012) found the opposite pattern, as individuals with AA or AG genotypes had higher OT levels than GG individuals. Other researchers have suggested that there may be population differences in the relationship between specific alleles such that each population has evolved independent relationships between alleles, hormone levels and human social behaviors (Costa et al., 2009; Ebstein et al., 2012). Along these lines, Ebstein et al. (2012) discussed the flip-flop effects of so-called risk alleles as the possible effect of different study populations. The previous study by Feldman et al. (2012) was conducted in Israel, whereas our study was conducted in Newfoundland, Canada (63.46% of the study population were native Newfoundlanders) which has a well-documented incidence of genetic isolation (Rahman et al., 2003). We suggest that further study of the Newfoundland population relative to other groups is necessary to tease apart potential population differences.

Men in the Provider group with the homozygous recessive GG genotype of *OXTR* rs53576 had higher OT levels than men with AA or AG genotypes. The GG genotype has been associated with greater sociability (Li et al., 2015), increased parental responsiveness (Bakermans-Kranenburg and van Ijzendoorn, 2008; Riem et al., 2011), as well as increased empathetic behaviors and reduced stress reactivity (Rodrigues et al., 2009), behaviors suggestive of individuals with higher OT. Similarly, Marsh et al., (2012) found that intranasal OT increased the preference for infant over adult pictures specifically in adults with the GG genotype. These results are also consistent with findings that men with the GG genotype show greater social competence (Krueger et al., 2012; Li et al., 2015; Weisman et al., 2015).

Our results for *CD38* support previous research that show higher OT levels for carriers of recessive CC genotypes compared to the AA and AC genotypes (Ebstein et al., 2012). In contrast, other studies have found higher OT levels in individuals with AA and AC than CC genotypes (Feldman et al., 2012), results that are consistent with research showing that AA and

AC individuals showed higher rates of parental touch (Feldman et al., 2012) and greater empathy to an individual in need (Liu et al., 2017). We suggest that the specific sociobehavioral context (i.e., peer-peer social interaction vs. partner/parenting contexts) may be the critical factor in the OT response of the individual (for a review see Lopatina et al., 2012).

There were no significant associations in our data for OT levels and *OXTR* 1042778. Previous research has often identified associations between *OXTR* 1042778 and characteristics of autism spectrum disorders (Campbell et al., 2011), sensitive parenting (Feldman et al., 2012) and empathetic romantic communication (Schneiderman et al., 2014). However, studies have also found null results similar to the current study (e.g., Liu et al., 2017). We suggest that, as with other OT receptor genes, there could be population differences in the links between specific genotypes and behavior and therefore replication of this study is needed to better understand that relationship between *OXTR* 1042778 and familial interactions.

Lastly, despite the structural similarity between OT and AVP and the presumed relation of AVP to partner and parenting behavior, we found no significant relationship between AVP and the 3-Group model.

4.3. RS-Partner/RS-Parent model

Although our results showed that AVP levels were related to an interaction between partner orientation and the number of *AVPR1A*-rs3 repeats, we did not find any significant relationships between the RS-Partner/RS-Parent model and *AVPR1A*-rs3. Our results differ from previous research that showed a relationship between *AVPR1A*-rs3 and partnering (Walum et al., 2008) and parenting behavior (Nishitani et al., 2017), but are consistent with findings of no relationship between *AVPR1A* and extra-pair mating (Zietsch et al., 2015). These contrasting results indicate the need for further research on AVP receptor microsatellites in relation to human reproductive behavior.

5. Conclusions

Overall, this study suggests that men's responses to partner and parental stimuli reflect how they experience the interaction, which in turn is affected by their hormone levels and their particular genotypes. Specifically, genetic characteristics may set the stage for a man's ideal reproductive strategy (i.e., Bachelor, Provider, Direct Father). Then due to natural variation in life's development and personal experience, men's hormones help modify behavior to allow them to successfully traverse their current context. For some men (i.e., Direct Father or Bachelor groups), their physiology and current context are in synch and require little change in response to partnering and parenting interactions. For other men (Providers), their physiology and current familial and sexual preferences remain more flexible and responsive to social cues. These results suggest that these men may have evolved the ability to maximize their reproductive success while minimizing the trade-offs often associated with choosing a narrow reproductive strategy. Future research should focus on expanding these results to include larger sample sizes and comparisons of genetically distinct populations, while carefully selecting the experimental interactions in order to capture the target effects for each hormone and genotype.

References

Acevedo, B.P., Aron, A., Fisher, H.E., Brown, L.L., 2012. Neural correlates of long-term intense romantic love. Soc. Cogn. Affect. Neurosci. 7, 145–159. https://doi.org/10.1093/scan/nsq092

Atzil, S., Hendler, T., Feldman, R., 2011. Specifying the neurobiological basis of human attachment: Brain, hormones, and behavior in synchronous and intrusive mothers. Neuropsychopharmacology 36, 2603–2615. https://doi.org/10.1038/npp.2011.172

Bakermans-Kranenburg, M.J., van Ijzendoorn, M.H., 2008. Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting. Soc. Cogn. Affect. Neurosci. 3, 128–134. https://doi.org/10.1093/scan/nsn004

Bartz, J.A., McInnes, L.A., 2007. CD38 regulates oxytocin secretion and complex social behavior. BioEssays 29, 837–841. https://doi.org/10.1002/bies.20623

Bell, V. C., Hughes, P., (2008). Signs – An Office Romance Short Film (Film Short). Australia. IndieFlix (Firm).

Caldwell, H.K., Lee, H.J., Macbeth, A.H., Young, W.S., 2008. Vasopressin: Behavioral roles of an "original" neuropeptide. Prog. Neurobiol. 84, 1–24. https://doi.org/10.1016/j.pneurobio.2007.10.007

Campbell, D.B., Datta, D., Jones, S.T., Lee, E.B., Sutcliffe, J.S., Hammock, E.A.D., Levitt, P., 2011. Association of oxytocin receptor (OXTR) gene variants with multiple phenotype domains of autism spectrum disorder. J. Neurodev. Disord. 3, 101–112. https://doi.org/10.1007/s11689-010-9071-2

Carson, D.S., Berquist, S.W., Trujillo, T.H., Garner, J.P., Hannah, S.L., Hyde, S.A., Sumiyoshi, R.D., Jackson, L.P., Moss, J.K., Strehlow, M.C., Cheshier, S.H., Partap, S., Hardan, A.Y., Parker, K.J., 2015. Cerebrospinal fluid and plasma oxytocin concentrations are positively correlated and negatively predict anxiety in children. Mol. Psychiatry 20, 1085–1090. https://doi.org/10.1038/mp.2014.132

Carson, D.S., Howerton, C.L., Garner, J.P., Hyde, S.A., Clark, C.L., Hardan, A.Y., Penn, A.A., Parker, K.J., 2014. Plasma vasopressin concentrations positively predict cerebrospinal fluid vasopressin concentrations in human neonates. Peptides 61, 12–16. https://doi.org/10.1016/j.peptides.2014.08.003

Cashdan, E., 1993. Attracting mates: Effects of paternal investment on mate attraction strategies. Ethol. Sociobiol. 14, 1–23. https://doi.org/10.1016/0162-3095(93)90014-9

Champagne, F.A., 2008. Epigenetic mechanisms and the transgenerational effects of maternal care. Front. Neuroendocrinol. 29, 386–397. https://doi.org/10.1016/j.yfrne.2008.03.003

Costa, B., Pini, S., Gabelloni, P., Abelli, M., Lari, L., Cardini, A., Muti, M., Gesi, C., Landi, S., Galderisi, S., Mucci, A., Lucacchini, A., Cassano, G.B., Martini, C., 2009. Oxytocin receptor polymorphisms and adult attachment style in patients with depression. Psychoneuroendocrinology 34, 1506–1514. https://doi.org/10.1016/j.psyneuen.2009.05.006

Ebstein, R.P., Knafo, A., Mankuta, D., Chew, S.H., Lai, P.S., 2012. The contributions of oxytocin and vasopressin pathway genes to human behavior. Horm. Behav. 61, 359–379. https://doi.org/10.1016/j.yhbeh.2011.12.014

Feldman, R., Gordon, I., Influs, M., Gutbir, T., Ebstein, R.P., 2013. Parental Oxytocin and Early Caregiving Jointly Shape Children ' s Oxytocin Response and Social Reciprocity 38, 1154–1162. https://doi.org/10.1038/npp.2013.22

Feldman, R., Gordon, I., Schneiderman, I., Weisman, O., Zagoory-Sharon, O., 2010. Natural variations in maternal and paternal care are associated with systematic changes in oxytocin following parent-infant contact. Psychoneuroendocrinology 35, 1133–1141. https://doi.org/10.1016/j.psyneuen.2010.01.013

Feldman, R., Zagoory-Sharon, O., Weisman, O., Schneiderman, I., Gordon, I., Maoz, R., Shalev, I., Ebstein, R.P., 2012. Sensitive parenting is associated with plasma oxytocin and polymorphisms in the OXTR and CD38 genes. Biol. Psychiatry 72, 175–181. https://doi.org/10.1016/j.biopsych.2011.12.025

Fernandez-Duque, E., Valeggia, C.R., Mendoza, S.P., 2009. The Biology of Paternal Care in Human and Nonhuman Primates. Annu. Rev. Anthropol. 38, 115–130. https://doi.org/10.1146/annurev-anthro-091908-164334

Fries, A.B.W., Ziegler, T.E., Kurian, J.R., Jacoris, S., Pollak, S.D., 2005. Early experience in humans is associated with changes in neuropeptides critical for regulating social behavior. Proc. Natl. Acad. Sci. U. S. A. 102, 17237–17240. https://doi.org/10.1073/pnas.0504767102

Gavrilets, S., 2012. Human origins and the transition from promiscuity to pair-bonding. Proc. Natl. Acad. Sci. U. S. A. 109, 9923–8. https://doi.org/10.1073/pnas.1200717109

Gettler, L.T., McDade, T.W., Feranil, A.B., Kuzawa, C.W., 2011. Longitudinal evidence that fatherhood decreases testosterone in human males. Pnas 108, 16194–16199. https://doi.org/10.1073/pnas.1105403108

Gordon, I., Zagoory-Sharon, O., Leckman, J.F., Feldman, R., 2010. Oxytocin and the development of parenting in humans. Biol. Psychiatry 68, 377–382. https://doi.org/10.1016/j.biopsych.2010.02.005

Grewen, K.M., Girdler, S.S., Amico, J., Light, K.C., 2005. Effects of Partner Support on Resting Oxytocin, Cortisol, Norepinephrine, and Blood Pressure Before and After Warm Partner Contact. Psychosom. Med. 67, 531–538. https://doi.org/10.1097/01.psy.0000170341.88395.47

Heim, C., Young, L.J., Newport, D.J., Mletzko, T., Miller, A.H., Nemeroff, C.B., 2009. Lower CSF oxytocin concentrations in women with a history of childhood abuse. Mol. Psychiatry 14, 954–958. https://doi.org/10.1038/mp.2008.112

Hostinar, C.E., Cicchetti, D., Rogosch, F.A., 2014. Oxytocin receptor gene polymorphism , perceived social support , and psychological symptoms in maltreated adolescents 26, 465–477. https://doi.org/10.1017/S0954579414000066

Inoue, T., Kimura, Ta., Azuma, C., Inazawa, J., Takemura, M., Kikuchi, T., Kubota, Y., Ogita, K., Saji, F., 1994. Structural Organization of the Human Oxytocin Receptor Gene. J. Biol. Chem. 269, 32451–32456. https://doi.org/10.1007/s00775-009-0595-4

Jin, D., Liu, H.X., Hirai, H., Torashima, T., Nagai, T., Lopatina, O., Shnayder, N.A., Yamada, K., Noda, M., Seike, T., Fujita, K., Takasawa, S., Yokoyama, S., Koizumi, K., Shiraishi, Y., Tanaka, S., Hashii, M., Yoshihara, T., Higashida, K., Islam, M.S., Yamada, N., Hayashi, K., Noguchi, N., Kato, I., Okamoto, H., Matsushima, A., Salmina, A., Munesue, T., Shimizu, N., Mochida, S., Asano, M., Higashida, H., 2007. CD38 is critical for social behaviour by regulating oxytocin secretion. Nature 446, 41–45. https://doi.org/10.1038/nature05526

Johnson, M., Sardi, J., Leven, J., Cassavetes, N., Gosling, R., McAdams, R., Garner, J., Alliance Atlantis (Firm). (2000). The notebook. Montreal: Alliance Atlantis.

Kelsey, T W, Lucy Q. Li, Rod T. Mitchell, Ashley Whelan, Richard A. Anderson, W.H.B.W., 2014. A validated age-related normative model for male total testosterone shows increasing variance but no decline after age 40 years: Editorial comment. J. Urol. 9, 109346. https://doi.org/10.1016/j.juro.2015.02.062

Krueger, F., Parasuraman, R., Iyengar, V., Thomburg, M., Weel, J., Lin, M., Clarke, E., McCabe, K., Lipsky, R., 2012. Oxytocin receptor genetic variation promotes human trust behavior 6, 1–9. https://doi.org/10.3389/fnhum.2012.00004

Landgraf, R., Neumann, I.D., 2004. Vasopressin and oxytocin release within the brain: A dynamic concept of multiple and variable modes of neuropeptide communication. Front. Neuroendocrinol. 25, 150–176. https://doi.org/10.1016/j.yfrne.2004.05.001

Li, J., Zhao, Y., Li, R., Broster, L.S., Zhou, C., Yang, S., 2015. Association of oxytocin receptor gene (OXTR) rs53576 polymorphism with sociality: A meta-analysis. PLoS One 10, 1–16. https://doi.org/10.1371/journal.pone.0131820
Liu, J., Gong, P., Li, H., Zhou, X., 2017. A field study of the association between CD38 gene and altruistic behavior: Empathic response as a mediator. Psychoneuroendocrinology 85, 165–171. https://doi.org/10.1016/j.psyneuen.2017.08.010
Lopatina, O., Inzhutova, A., Salmina, A.B., Higashida, H., 2012. The roles of oxytocin and CD38 in social or parental behaviors. Front. Neurosci. 6, 1–12. https://doi.org/10.3389/fnins.2012.00182
Love, T., 2015. Oxytocin, Motivation and the Rold of Dopamine. Nih 49–60. https://doi.org/10.1016/j.pbb.2013.06.011.Oxytocin
Lucassen, N., Tharner, A., Van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Volling, B.L., Verhulst, F.C., Lambregtse-Van den Berg, M.P., Tiemeier, H., 2011. The Association Between Paternal Sensitivity and Infant-Father Attachment Security: A Meta-Analysis of Three Decades of Research. J. Fam. Psychol. 25, 986–992. https://doi.org/10.1037/a0025855
Luo, S., Li, B., Ma, Y., Zhang, W., Rao, Y., Han, S., 2015. Oxytocin receptor gene and racial ingroup bias in empathy-related brain activity. Neuroimage 110, 22–31. https://doi.org/10.1016/j.neuroimage.2015.01.042
MacQueen, J., 1967. Some Methods for classification and Analysis of Multivariate Observations. 5th Berkeley Symp. Math. Stat. Probab. 1967 1, 281–297. https://doi.org/citeulike-article-id:6083430
Marazziti, D., Canale, D., 2004. Hormonal changes when falling in love. Psychoneuroendocrinology 29, 931–936. https://doi.org/10.1016/j.psyneuen.2003.08.006
Marks, L., Palkovitz, R., 2004. American Fatherhood Types: The Good, the Bad, and the Uninterested. Father. A J. Theory, Res. Pract. about Men as Father. 2, 113–129. https://doi.org/10.3149/fth.0202.113
Marsh, A., Yu, H., Pine, D., Gorodetsky, E., Goldman, D., Blair, J., 2012. The Influence of Oxytocin Administration on Responses to Infant Faces and Potential Moderation by OXTR Genotype. Psychopharmacology (Berl). 224, 469–476. https://doi.org/10.1007/s00213-012-2775-0.The
Mascaro, J.S., Hackett, P.D., Gouzoules, H., Lori, A., Rilling, J.K., 2014. Behavioral and genetic correlates of the neural response to infant crying among human fathers. Soc. Cogn. Affect. Neurosci. 9, 1704–1712. https://doi.org/10.1093/scan/nst166
McCullough, M.E., Churchland, P.S., Mendez, A.J., 2013. Problems with measuring peripheral oxytocin: Can the data on oxytocin and human behavior be trusted? Neurosci. Biobehav. Rev. 37, 1485–1492. https://doi.org/10.1016/j.neubiorev.2013.04.018
Melis, M.R., Argiolas, A., 2011. Central control of penile erection: A re-visitation of the role of oxytocin and its interaction with dopamine and glutamic acid in male rats. Neurosci. Biobehav. Rev. 35, 939–955. https://doi.org/10.1016/j.neubiorev.2010.10.014
Miller, S.A., Gallie, D.R., Sleat, D.E., Watts, J.W., Turner, P.C., Wilson, T.M. a, 1988. A Simple Salting Out Procedure for Extracting DNA from Human Nucleated Cells. Nucleic Acids Res. 16, 883–893.
Neumann, I.D., 2008. Brain oxytocin: A key regulator of emotional and social behaviours in both females and males. J. Neuroendocrinol. 20, 858–865. https://doi.org/10.1111/j.1365-2826.2008.01726.x
Nishitani, S., Ikematsu, K., Takamura, T., Honda, S., Yoshiura, K. ichiro, Shinohara, K., 2017. Genetic variants in oxytocin receptor and arginine-vasopressin receptor 1A are associated with the neural correlates of maternal and paternal affection towards their child. Horm. Behav. 87, 47–56. https://doi.org/10.1016/j.yhbeh.2016.09.010

Prichard, Z.M., Mackinnon, A.J., Jorm, A.F., Easteal, S., 2007. AVPR1A and OXTR polymorphisms are associated with sexual and reproductive behavioral phenotypes in humans. Mutation in brief no. 981. Online. Hum. Mutat. 28, 1150. https://doi.org/10.1002/humu.9510

Rahman, P., Jones, A., Curtis, J., Bartlett, S., Peddle, L., Fernandez, B.A., Freimer, N.B., 2003. The Newfoundland population: a unique resource for genetic investigation of complex diseases. Hum. Mol. Genet. 12, R167–R172. https://doi.org/10.1093/hmg/ddg257

Riem, M.M.E., Bakermans-Kranenburg, M.J., Pieper, S., Tops, M., Boksem, M.A.S., Vermeiren, R.R.J.M., Van Ijzendoorn, M.H., Rombouts, S.A.R.B., 2011. Oxytocin modulates amygdala, insula, and inferior frontal gyrus responses to infant crying: A randomized controlled trial. Biol. Psychiatry 70, 291–297. https://doi.org/10.1016/j.biopsych.2011.02.006

Rodrigues, S.M., Saslow, L.R., Garcia, N., John, O.P., Keltner, D., 2009. Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. Proc. Natl. Acad. Sci. 106, 21437–21441. https://doi.org/10.1073/pnas.0909579106

Scheele, D., Striepens, N., Gunturkun, O., Deutschlander, S., Maier, W., Kendrick, K.M., Hurlemann, R., 2012. Oxytocin Modulates Social Distance between Males and Females. J. Neurosci. 32, 16074–16079. https://doi.org/10.1523/JNEUROSCI.2755-12.2012

Schneiderman, I., Kanat-maymon, Y., Ebstein, R.P., Feldman, R., 2014. Cumulative risk on the oxytocin receptor gene (OXTR) underpins empathic communication difficulties at the first stages of romantic love. Soc. Cogn. Affect. Neurosci. 9, 1524–1529. https://doi.org/10.1093/scan/nst142

Spanier, G.B., 1976. Measuring Dyadic Adjustment: New Scales for Assessing the Quality of Marriage and Simialr Dyads. J. Marriage Fam.

Sroufe, L.A., 2005. Attachment and development: A prospective, longitudinal study from birth to adulthood. Attach. Hum. Dev. 7, 349–367. https://doi.org/10.1080/14616730500365928

Steiner, E.T., 2011. Testosterone and vasopressin in men ' s reproductive behavior. University of Nevada.

Strathearn, L., Fonagy, P., Amico, J., Montague, R., 2011. To Infant Cues. Read 34, 2655–2666. https://doi.org/10.1038/npp.2009.103.Adult

Tansey, K.E., Hill, M.J., Cochrane, L.E., Gill, M., Anney, R.J.L., Gallagher, L., 2011. Functionality of promoter microsatellites of arginine vasopressin receptor 1A (AVPR1A): implications for autism. Mol. Autism 2, 3. https://doi.org/10.1186/2040-2392-2-3

Thibonnier, M., Graves, M.K., Wagner, M.S., Auzan, C., Clauser, E., Willard, H.F., 1996. Structure, sequence, expression, and chromosomal localization of the human V1a vasopressin receptor gene. Genomics 31, 327–334. https://doi.org/10.1006/geno.1996.0055

Valstad, M., Alvares, G.A., Egknud, M., Matziorinis, A.M., Andreassen, O.A., Lars, T., Quintana, D.S., 2016. The correlation between central and peripheral oxytocin concentrations: a systematic review and meta-analysis Mathias.

van Anders, S.M., Tolman, R.M., Volling, B.L., 2012. Baby cries and nurturance affect testosterone in men. Horm. Behav. 61, 31–36. https://doi.org/10.1016/j.yhbeh.2011.09.012

Veening, J.G., de Jong, T., Barendregt, H.P., 2010. Oxytocin-messages via the cerebrospinal fluid: Behavioral effects; a review. Physiol. Behav. 101, 193–210. https://doi.org/10.1016/j.physbeh.2010.05.004

Wallen, K., 2001. Sex and context: Hormones and primate sexual motivation. Horm. Behav. 40, 339–357. https://doi.org/10.1006/hbeh.2001.1696

Walum, H., Lichtenstein, P., Neiderhiser, J.M., Reiss, D., Ganiban, J.M., Spotts, E.L., Pedersen, N.L., Anckarster, H., Larsson, H., Westberg, L., 2012. Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. Biol. Psychiatry 71, 419–426. https://doi.org/10.1016/j.biopsych.2011.09.002

Walum, H., Westberg, L., Henningsson, S., Neiderhiser, J.M., Reiss, D., Igl, W., Ganiban, J.M., Spotts, E.L., Pedersen, N.L., Eriksson, E., Lichtenstein, P., 2008. Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. Proc. Natl. Acad. Sci. U. S. A. 105, 14153–14156. https://doi.org/10.1073/pnas.0803081105

Weisman, O., Pelphrey, K.A., Leckman, J.F., Feldman, R., Lu, Y., Chong, A., Chen, Y., Monakhov, M., Chew, S.H., Ebstein, R.P., 2015. The association between 2D:4D ratio and cognitive empathy is contingent on a common polymorphism in the oxytocin receptor gene (OXTR rs53576). Psychoneuroendocrinology 58, 23–32. https://doi.org/10.1016/j.psyneuen.2015.04.007

Wilhelm, K., Parker, G., 1988. The Development of a Measure of Intimate Bonds. Psychol. Med. 18, 225–234.

Zietsch, B.P., Westberg, L., Santtila, P., Jern, P., 2015. Genetic analysis of human extrapair mating: Heritability, between-sex correlation, and receptor genes for vasopressin and oxytocin. Evol. Hum. Behav. 36, 130–136. https://doi.org/10.1016/j.evolhumbehav.2014.10.001

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

Chapter 3

Correlates of Cortisol and Male Reproductive Strategies: Relationships between Cortisol, Testosterone, Oxytocin, Vasopressin, and Male Reproductive Behaviors

by

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Abstract

Studies of the complex and context-specific effects of cortisol (CORT) have resulted in mixed conclusions concerning male partnering and parenting behavior. To better understand the relationship(s) between CORT and the hormones T, OT, and AVP, I examined the behavioral responses of men in relation to hormone levels and hormone ratios in the context of a partnering and a parenting challenge. Fifty-two men (19-45 years old) answered questionnaires and provided blood samples before and after interacting in two experimental sessions. Sessions occurred (counterbalanced) approximately one week apart and included viewing a romantic video (two film clips watched with partner, if applicable) and a paternal caregiving interaction (RealCare baby doll). K-mean clustering analyses of demographic data, including health and family history, as well as relationship status, created two statistical models for evaluation: The 3-Group model (Bachelors, Providers and Direct Fathers) and the RS-Partner/RS-Parent model (high vs. low partner/parent). CORT levels decreased significantly more in the baby doll session than in the video session, suggesting that arousal decreased in the parenting but not the partnering context. T/CORT, OT/CORT, and AVP/CORT ratios increased over the baby doll session and were highest in the baby doll session for the least involved men (low partner/low parent group), suggesting that these men are least prone to the higher arousal associated with infant care.

Keywords: Cortisol, Testosterone, Oxytocin, Vasopressin, Reproductive Strategies, Sociobehavioral Endocrinology, Paternal Care, Hormone Ratios.

Highlights:

- In two models of parenting/partner investment, men showed CORT decreases after interacting with a simulated baby
- Ratios of T, OT, and AVP relative to CORT increased in men after interacting with the doll vs. watching a romantic video
- In the RS/RS model, men in the low parent group showed decreased OT during the baby doll session
- Men in low parent/low partner group showed significantly higher ratios of T, OT, and AVP relative to CORT than other men
- In the 3-Group model, Bachelors had higher T/OT and T/AVP ratios than Providers or Direct Fathers

1. Introduction

Cortisol (CORT) has often been included in studies of human partnering and parenting focusing on testosterone (T) and/or oxytocin (OT), but until recently rarely as the hormone of primary interest or as a focus in partner studies. A difficulty with interpreting CORT responses is that they appear to depend on the specifics of the social and behavioral context. For example, men experience elevated CORT levels during stressful situations (Kirschbaum et al., 1995, reviewed in Erickson et al., 2003), as well as in situations of more positive arousal such as the early phases of romantic relationships (Marazziti and Canale, 2004), and just before the birth of their babies (Berg and Wynne-Edwards, 2001; Storey et al., 2000). Results are also mixed for the relationship between paternal investment and CORT levels: greater paternal involvement has been linked to both higher (Kuo et al., 2018), lower (Bos et al., 2018), and declining CORT levels (Gettler et al., 2011). This complex pattern of results highlights the importance of examining CORT responses in specific contexts.

1.1 Cortisol & Testosterone

Little is known about how CORT interacts with other hormones but recent theoretical developments may help to guide research questions. The Dual Hormone Hypothesis posits that the role of T in status-related social contexts is dependent on concentrations of CORT (Dekkers et al., 2019; Mehta and Prasad, 2015). Specifically, T levels should increase when the individual participates in status-seeking activities, but only when CORT levels are low (Mehta and Prasad, 2015). For example, higher T was associated with decreased self-reported empathy among individuals with low CORT (Zilioli et al., 2015). Furthermore, elevated CORT levels may effectively block or inhibit the actions of T. If transferable to parenting and partnering situations, similar results might be predicted for mating competition (i.e., men with high T and low CORT being more successful). However, for parenting situations, wherein low T levels are generally associated with more effective care, the question would be whether high (more arousal and focus) or low (less stress) CORT levels would result in more effective care. We will examine whether variation in hormones other than T may also be affected by variation in CORT levels.

Increases in T and CORT have been implicated in both mating and parenting behavior. T and CORT increased following a brief interaction with a potential mate, particularly in men with fewer repeats at their androgen receptor gene and lower baseline CORT levels (Roney et al., 2010). These results suggest that the CORT increases during mating interactions may prepare males for the energy demands of courtship and possible parenting (for review Erickson et al., 2003). A study of subsistence hunters found increases in T and CORT levels in association with a successful hunt (Trumble et al.,

2013), likely due to the physical exertion of the hunt. In this case, it was timing of the increases (at the time of the kill rather than at social recognition when they returned to the village) that led authors to suggest that success of male provisioning was the driving force behind the hormone changes rather than male-competition. However, men have also been found to be more socially successful in groups when T levels are high and CORT levels are low (Ponzi et al., 2016). The contrasting interpretations from these studies highlight the subtleties and difficulties in linking male reproductive roles to changes in hormone levels (see Carré and Olmstead, 2015 for review).

1.2. Cortisol & Oxytocin

CORT and oxytocin (OT) have both been implicated in social interactions, familial bonding, and stress responses. A study of interactions between mother, father, and infant found positive triadic synchrony to be predicted by higher OT and lower CORT levels in mothers and fathers. The authors note that CORT levels were elevated in mothers who reported low direct care by the fathers (Gordon et al., 2010). By extension, OT and CORT have been shown to be related to bonding and attachment through the anxiety associated with managing and maintaining close bonds (Gordon et al., 2008). The combined effects of OT and social support appear to suppress the CORT response to psychosocial stress (Heinrichs et al., 2003). These data indicate the value of evaluating the ratios of CORT and OT in the context of both parenting and partnering.

1.3. Cortisol & Vasopressin

To my knowledge, there are no studies to date that directly evaluate the relationship between CORT and AVP though studies have independently examined the relationships between these hormones, social interactions, and stress. Studies of parenting

and partnering have often included AVP when examining OT as the neuropeptides are so similar and there is potential for cross-activation of associated receptors. Higher levels of endogenous AVP levels have been associated with a higher proportion of experimental session time spent in stimulatory play between fathers and their infants (Apter-Levi et al., 2014). Additionally, in a virtual reality experiment intranasal administration of AVP increased interest in avatars relating to parenting (Cohen-Bendahan et al., 2015). There appears to be an intergenerational component to AVP effects: men who reported a history of paternal warmth showed greater empathy in response to distressing images after intranasal AVP than men not reporting paternal warmth (Tabak et al., 2015).

1.4. Theoretical Models

To better understand the relationships between hormones and their behavioral context, researchers have developed new theoretical frameworks yielding interesting perspectives. For example, the Steroid-Peptide Theory (S/P Theory) states that T is related to tradeoffs in social bonding and parental care, while the neurotransmitters OT and AVP are positively tied to social bonding (van Anders et al., 2011). The authors theorize two models of pair and parent-infant bonds: a singular model in which there is a shared evolutionary history for both types of bonds, or a bimodal model in which sexual and nurturant intimacy can elicit divergent hormone responses. Furthermore, the S/P Theory differentiates between hormone predictions based on the context and subjective experience of the individual (e.g., sexual intimacy increases T and OT, while nurturant intimacy decreases T and increases OT). Therefore, T levels will likely increase following the interactions with the partner, particularly in the men oriented toward partnership rather than parenting. Further, CORT levels have been shown to increase during social

intimacy (Smith et al., 2009), but decrease during sexual intimacy (van Anders and Gray, 2007), suggesting that CORT could decrease during partner interactions depending on the subjective experience of the interaction.

This study will build on previous research of male reproductive behaviors and physiology by evaluating the relationships between the hypothesized reproductive models: 3-Group model and RS/RS model (see Chapter 1 for behavioral grouping results). The 3-Group model divides men into three categories based on reported characteristics of both partnering and parenting variables: Bachelor, Provider, and Direct Father. Thus, the model deals with individual variability in both parenting and partnering in the context of the van Anders et al. (2011) suggestion that both forms of intimacy may share an evolutionary history. In contrast, the RS-Partnering/RS-Parenting model separates partnering and parenting variables to create distinct, but interrelated, categories of individuals, with men falling into High/Low Partnering and High/Low Parenting groups, as per the van Anders et al. (2011) alternate suggestion that these two systems may have evolved independently, with partner behaviors occurring independently from parenting behaviors.

1.5. Analyses

Analyses of complex theoretical models involving multiple hormones can be difficult. Some researchers have chosen to utilize hormone ratios (Sollberger and Ehlert, 2016) as a means of analyzing and interpreting multiple hormones in context of social behavior. For example, men who had committed intimate partner violence had higher T/CORT ratios than other men (Romero-Martínez et al., 2013). In general, this method can be informative but problematic as the hormone relationship can be difficult to

accurately represent in relation to each other (i.e., which hormone should be the numerator and denominator). Likewise, the distribution of the hormone data needs to be taken into account (i.e., normality). In a similar type of study, Sollberger et al. (2015) evaluated the relationship between T/CORT and pro-environmental behavior using hormone ratios and used log transformations to normalize their data (which was not necessary in the current study). Results indicated a negative relationship between T and pro-environmental behavior but only in men with low CORT. Authors warned that accurate interpretation of the results was critical and stressed that need for further study to identify other possible influences on the outcome variable. For the purposes of this study, I chose to utilize hormone ratios based on the normal distribution of data with reference to the body of research indicating the likely directions of relation between the focal hormones (with CORT as the denominator).

1.6. Objectives

This study aimed to examine the relationships between CORT and the hormones often associated with partnering and parenting behaviors: T, OT, and AVP. Examining CORT in relation to other hormones may allow a more precise characterization of individual differences in responses to parenting and partnering contexts. Consistent with previous literature (e.g., Mehta and Prasad, 2015; Zilioli et al. , 2015), low or decreasing CORT levels should allow the behavioral effects of the other hormones to be most apparent; whereas high or increasing CORT may modulate those effects. CORT is expected to decrease if participants become more comfortable in the experimental session (Storey et al., 2000) or if the sessions are perceived to be low stress. In contrast, CORT is expected to increase if sessions increase arousal or focus. T/CORT ratios should therefore

increase or be high for the less parent-oriented men in the baby doll session (T stable or increasing/CORT decreasing), as these men would be less interested in such interactions. Alternatively, T/CORT ratios should be low or decreasing for more parent-oriented men (T stable or decreasing/CORT stable or increasing). OT/CORT and AVP/CORT ratios are expected to increase more or decrease less for partner and parent-oriented men than the other men.

2. Methods

2.1. Study Population

A total of 52 men were recruited from prenatal, breastfeeding, and infant care classes at the Women's Health Centre located in the Health Sciences Centre, St. John's, Newfoundland and Labrador. Participants were also recruited through informational talks and interdepartmental newsletters at Memorial University of Newfoundland, as well as through community flyers, posters, and blood donor clinics. Institutional approval from Human Research Ethics Authority (Memorial University of Newfoundland – 13.105) was obtained prior to data collection for this study on August 22, 2013. There was a total of 44 men in the partner video session and 46 men in the baby doll session and 38 of the men participated in both the video and the doll session. All men were English speaking and between the ages of 19 and 45 years old ($M = 31.24 \pm 7.313$). Participant ages were selected to avoid the hormonal peaks following the end of puberty and the declines of aging males (Kelsey et al., 2014). Participants self-reported their place of origin as Newfoundland (n=33), other parts of Canada (n=11), or other (n=8). Participants reported their parental status as having a baby (n=11), having older children (n=12), or no children

(n=29). Of interest, of the men that reported not having children, 17 reported wanting children in the future, while 12 men reported not wanting children.

2.2. Procedure

Men participated in between one to two experimental sessions in the Psychology Department of Memorial University, approximately 1-week apart at approximately 1200-1600 between December, 2013 and May, 2015. Participants were asked to refrain from caffeine, alcohol, and sexual interaction for the 24-hours prior to their session. Participants were also asked to refrain from intense exercise just prior to the session. During the sessions, participants filled out questionnaires related to personal demographics, relationship dynamics, interactions with children, and their experiences during the experimental sessions. Once the questionnaires were complete, I took a venous blood sample for hormone analyses. Following blood sampling, participants interacted with a robotic baby doll (RealCare baby manufactured by RealityWorks - see van Anders et al., 2012) or watched a pair of romantic videos with their partner (if applicable). Approximately 30-mins after the first blood sample, participants gave a second blood sample and reported their experiences during the session. Partners were present throughout the experimental session.

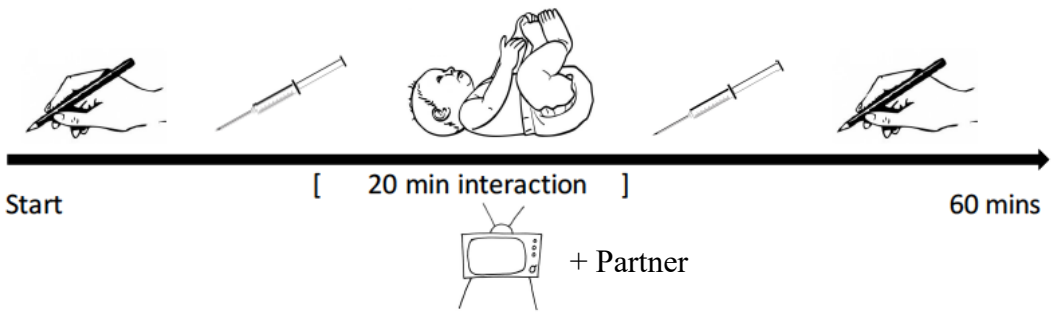

Figure 1. Experimental method for romantic video session and RealCare baby doll session.

* The second blood sample was taken approximately 30-mins after the first blood sample.

2.3. Questionnaire data

Questionnaires included demographic information, the Dyadic Adjustment Scale (DAS- Spanier, 1976), the Intimate Bond Measure (IBS –Wilhelm & Parker, 1988), and the post-interaction questionnaire (see Appendices). The demographic information questionnaire covered topics such as age, weight, smoking habits, profession, place of origin, relationship status, parenting experience, and sexual relations and history. Variables, such as "Time with Kids", were interpreted from the raw demographic data. For example, "Time with Kids" was categorized by high (≥10 hours per week) or low (<10 hours per week) interaction (defined as being primarily responsible for child's care) with children.

The DAS included thirty-two scaled questions (i.e., 0 =Always Disagree, 5= Always Agree; "Which of the following statements best describes…"; "yes" or "no" questions) related to interaction quality across relationship topics. Scoring for the DAS is broken down into four categories: perception, affection, consensus, and cohesion, as well

as the total score (described in detail in Spanier, 1976). Although the scoring method for the DAS includes these five interdependent subcategories, we decided to use only the total DAS score as a representation of relationship quality for our statistical analyses (as per Walum et al., 2008).

The IBS included 24 questions regarding partner interaction quality (Wilhelm & Parker, 1988). Twelve of the questions relate to care behaviors and twelve of the questions relate to control behaviors. For example, participants responded to the statement "my partner wants to know where I am at all times". Answers were rated as "true", "moderately", "somewhat", or "not at all", with higher numbers representing higher levels of care and/or control in the relationship.

Behavioral data for all participants were examined for patterns in questionnaire data by K-means clustering analyses. Cluster analyses was selected for three groups based on partnering and parenting behaviors together (3-Group model: Bachelor, Provider, Direct Father) and then for the alternative hypothesis that parental and pair-bonding behaviors are not mutually exclusive, meaning that an individual could be high on one or both categories, or neither (RS-Partner/RS-Parent model: high vs. low partner and parent).

2.4. Pair-bonding interaction

During the video session each man watched two romantic videos (one sexually romantic and one emotionally romantic) with his partner. The sexually romantic video was an excerpt from The Notebook (2004 – PG-13), depicting two estranged lovers reuniting in a mildly sexual scene. The emotionally romantic video was a short film titled Signs (2008), depicting an office romance at a distance with the use of "signs" (sheets of

paper with short notes on them). All session were approximately 60-minutes long, with approximately 30-minutes between blood samples, and they occurred in the same laboratory setting.

2.5. RealCare baby doll interaction

The RealCare baby (RealityWorks, Eau Claire, WI, USA) approximated a 6-month old male infant (as used in van Anders, Tolman, & Volling, 2012). It was dressed in a blue shirt and footed pants. Once activated, the doll behaved as directed by the same selected care program for each participant. During the 20-minute interaction the doll 'requested' four care events: bottle feed, burp, diaper change, and gentle rocking. Each man was responsible for identifying and providing the care required. The doll recorded successful vs. unsuccessful care attempts (percentage out of 100), number of minutes spent crying, and any instances of rough handling (deducted points from total). Data from the doll was downloaded upon completion of the interaction and participants were asked to fill-out a follow-up questionnaire about their experiences during the session. The doll has not been formally validated but the change in OT in a small sample of new fathers tested with their own babies in this study was marginally correlated with their OT change with the baby doll ($r = 0.84$, $p = .08$, $n = 5$).

2.6. Sample collection and processing protocol

Blood samples were collected by standard protocol for venous blood collection into 8ml EDTA filled plastic tubes. Samples were kept on ice for up to one hour and then centrifuged at 1,6000g for 15-mins. Once centrifuged, plasma was aliquoted into a 2-ml O-ring tubes and stored at 4°C for 1-3 months prior to hormone analyses. The remaining sample (white and red blood cells) was stored at 4°C for up to 1-week for DNA extraction

in batches by salting out method (Miller et al., 1988). Extracted DNA samples were frozen at 20°C for genotyping.

2.7. Cortisol and testosterone assay

Cortisol and testosterone immunoassays (nmol/mL) were performed in duplicate at the clinical laboratory at the Health Sciences Hospital at Memorial University, St. John's, Newfoundland. Plasma samples were run on Abbott Diagnostics Architect; 2000SR immunoassay analyzer with a 2nd Generation Cortisol or Testosterone assay kit, following standard protocol as described in the kit inserts. The within and between run imprecision for serum cortisol and testosterone using this method is generally less than 5 percent for samples between 0.5 and 46.0 nmol/L. Run imprecision is validated regularly at this laboratory for all clinical assays, including these samples.

2.8. OT and AVP assay

OT and AVP enzyme immunoassays (pg/mL) were performed at the Pollak Laboratory at McGill University, Montreal, Canada. Plasma samples were processed with an Enzo Life Sciences assay kits (Oxytocin ELISA kit Catalog No. ADI-900-153; arg8-Vasopressin EIA kit Catalog No. ADI-900-017), following standard protocol as described in the kit insert. Samples were run in duplicate, with the average of the two used for the final data point; intra-assay coefficient of variation values were calculated as an average of 2.84% for OT and 3.52% for AVP.

2.9. Statistical Methods

All statistical analyses were performed in SPSS version 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Results were considered significant at $p<0.05$. Two types of analyses were conducted:

1. *Single hormone analysis*. General linear modeling (GLM) was used to compare the differences in the change between the two experimental sessions (baby doll and video, repeated measures independent variable) in each hormone (dependent measure). Group membership was the between subjects independent variable in each of the two models, the 3-group model (one variable with three levels: Bachelor, Provider, Direct Father) and the RS-Parent/RS-Partner model (two variables each with two levels: high and low).

2. *Hormone ratios analyses*. General linear modeling (GLM) was used to compare ratios before and after each experimental session (repeated measures independent variable). Group membership was the between subjects independent variable in each of the two models, the 3-group model (one variable with three levels) and the RS-Parent/RS-Partner model (two variables each with two levels). Five hormone ratios (T/CORT, OT/CORT, AVP/CORT, T/OT, T/AVP) were tested with each of the two models and in each of the two experimental sessions (baby doll and video).

3. Results

3.1. Single Hormone Analyses

CORT levels decreased significantly more in the baby doll session than in the video session in both the 3-Group model ($F_{1,34} = 6.39$, $p = 0.02$, $eta^2 = .16$, Power =.69) and the RS-Partner/RS-Parent analyses ($F_{1,33} = 6.73$, $p = 0.02$; $eta^2 = .17$, Power =.71; Figure 1).

The Group factor was not significant in either analysis (p = 0.23 – 0.65, eta^2 = .04 -.05, Power = .07 - .22), nor were the interactions (p = 0.11 - 0.98, eta^2 = .11-.12, Power = .38.-.44).

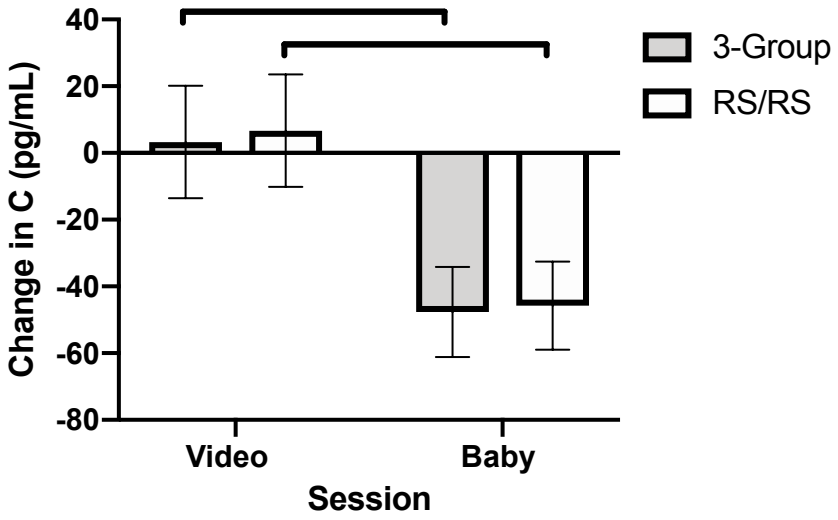

Figure 2. CORT levels (nmol/mL; M±SE) decreased more during the baby doll session than during the video session in both the 3-Group and the RS/RS analyses (N = 37).

The change in OT levels did not differ between the video and baby doll sessions in the 3-group ($F_{1,33}$ = 0.05, p = 0.82; eta^2 = <.01, Power = .06) and RS partner-RS parent analyses ($F_{1,33}$ = 0.01, p = 0.95; eta^2 = .001, Power = .05) and there were no significant interactions. There was a significant RS-Parent effect with average overall OT levels decreasing significantly more in men in the low parent group than men in the high parent group ($F_{1,32}$ = 4.58, p = 0.04, eta^2 = .13, Power = .55;Table 1). The sample size was larger here than in the overall analysis with the OXTR receptor genes in chapter 2, and those analyses dealt with hormone levels (chapter 2) rather than change (chapter 3) and these

factors may explain what appear to be different outcomes. There were no other significant changes in OT for session or Group in either analysis.

Similarly, changes in T or AVP levels did not differ between the video and baby doll in the 3-Group model (T, eta^2 = 0.001, Power = .05; AVP, eta^2 = .002, Power = .06) or RS-Partner and RS-Parent model (T, eta^2 = .001, Power = .05; AVP, eta^2 = .10, Power = .44)

Table 1. Significant main effects for hormone changes by factor.

Hormone	Factor	N	Mean ± SE	P
CORT	Baby	37	-47.633 ± 13.491	0.02
	Video	37	3.290 ± 16.838	
OT - RS Parent	Low Parent	12	-47.625 ± 24.509	0.04
	High Parent	24	13.033 ± 14.210	

Correlation analyses indicates some strong relationships between hormone levels over time and between sessions, and in some cases between hormones. Results indicate strong positive correlations for T across and between sessions (Table 2). Similar results were also shown for OT. CORT was highly positively correlated over the baby session, but not the video session. Lastly, AVP levels were highly positively correlated across and between sessions, as well as with some measures of OT.

Table 2. Correlations among hormones. Variables listed as hormone (T, CORT, OT, AVP), before/after (b/a), and session (2=video, 3=baby doll).

		tb2	ta2	cortb2	corta2	otb2	ota2	avpb2	avpa2	tb3	ta3	cortb3	corta3	otb3	ota3	avpb3	avpa3
tb2	Pearson	1	.969**	0.109	0.149	0.104	-0.025	-0.273	-0.192	.748**	.709**	-0.01	0.067	-0.054	0.18	-0.202	-0.107
	Sig.		0	0.487	0.339	0.505	0.874	0.076	0.223	0	0	0.951	0.692	0.749	0.286	0.23	0.53
	N	43	43	43	43	43	42	43	42	37	37	37	37	37	37	37	37
ta2	Pearson	.969**	1	0.13	0.123	0.217	0.075	-0.227	-0.177	.751**	.716**	0.083	0.139	-0.042	0.185	-0.173	-0.048
	Sig.	0		0.406	0.432	0.161	0.639	0.144	0.261	0	0	0.627	0.411	0.804	0.273	0.307	0.778
	N	43	43	43	43	43	42	43	42	37	37	37	37	37	37	37	37
cortb2	Pearson	0.109	0.13	1	.459**	-0.057	-0.135	0.234	0.257	0.204	0.137	0.292	0.247	0.029	-0.05	-0.006	0.064
	Sig.	0.487	0.406		0.002	0.717	0.394	0.13	0.1	0.225	0.419	0.079	0.141	0.863	0.771	0.971	0.707
	N	43	43	43	43	43	42	43	42	37	37	37	37	37	37	37	37
corta2	Pearson	0.149	0.123	.459**	1	-0.077	0.053	0.003	0.238	0.161	0.16	0.069	0.078	-0.11	-0.084	0.189	0.084
	Sig.	0.339	0.432	0.002		0.624	0.737	0.984	0.13	0.34	0.343	0.687	0.647	0.518	0.621	0.263	0.619
	N	43	43	43	43	43	42	43	42	37	37	37	37	37	37	37	37
otb2	Pearson	0.104	0.217	-0.057	-0.077	1	.805**	0.177	0.243	0.062	0.128	-0.017	-0.029	.609**	.606**	-0.041	0.071
	Sig.	0.505	0.161	0.717	0.624		0	0.255	0.122	0.714	0.449	0.921	0.867	0	0	0.81	0.678
	N	43	43	43	43	43	42	43	42	37	37	37	37	37	37	37	37
ota2	Pearson	-0.025	0.075	-0.135	0.053	.805**	1	0.271	.384*	0.058	0.169	0.021	0.013	.636**	.562**	0.27	.347*
	Sig.	0.874	0.639	0.394	0.737	0		0.083	0.012	0.736	0.324	0.904	0.941	0	0	0.111	0.038
	N	42	42	42	42	42	42	42	42	36	36	36	36	36	36	36	36
avpb2	Pearson	-0.273	-0.227	0.234	0.003	0.177	0.271	1	.838**	-0.144	-0.173	0.003	-0.072	.454**	0.218	.611**	.554**
	Sig.	0.076	0.144	0.13	0.984	0.255	0.083		0	0.396	0.305	0.988	0.673	0.005	0.195	0	0
	N	43	43	43	43	43	42	43	42	37	37	37	37	37	37	37	37
avpa2	Pearson	-0.192	-0.177	0.257	0.238	0.243	.384*	.838**	1	-0.057	-0.068	-0.058	-0.217	.436**	0.279	.658**	.539**
	Sig.	0.223	0.261	0.1	0.13	0.122	0.012	0		0.743	0.694	0.735	0.205	0.008	0.1	0	0.001
	N	42	42	42	42	42	42	42	42	36	36	36	36	36	36	36	36
tb3	Pearson	.748**	.751**	0.204	0.161	0.062	0.058	-0.144	-0.057	1	.935**	0.205	0.234	0.075	0.223	0.046	0.125
	Sig.	0	0	0.225	0.34	0.714	0.736	0.396	0.743		0	0.176	0.122	0.626	0.141	0.766	0.412
	N	37	37	37	37	37	36	37	36	45	45	45	45	45	45	45	45
ta3	Pearson	.709**	.716**	0.137	0.16	0.128	0.169	-0.173	-0.068	.935**	1	0.175	0.208	0.139	0.282	0.034	0.12
	Sig.	0	0	0.419	0.343	0.449	0.324	0.305	0.694	0		0.249	0.17	0.362	0.06	0.827	0.431
	N	37	37	37	37	37	36	37	36	45	45	45	45	45	45	45	45
cortb3	Pearson	-0.01	0.083	0.292	0.069	-0.017	0.021	0.003	-0.058	0.205	0.175	1	.781**	-0.033	-0.105	-0.131	-0.041
	Sig.	0.951	0.627	0.079	0.687	0.921	0.904	0.988	0.735	0.176	0.249		0	0.827	0.491	0.392	0.79
	N	37	37	37	37	37	36	37	36	45	45	45	45	45	45	45	45
corta3	Pearson	0.067	0.139	0.247	0.078	-0.029	0.013	-0.072	-0.217	0.234	0.208	.781**	1	-0.036	0.022	-0.058	0.092
	Sig.	0.692	0.411	0.141	0.647	0.867	0.941	0.673	0.205	0.122	0.17	0		0.815	0.887	0.706	0.547
	N	37	37	37	37	37	36	37	36	45	45	45	45	45	45	45	45
otb3	Pearson	-0.054	-0.042	0.029	-0.11	.609**	.636**	.454**	.436**	0.075	0.139	-0.033	-0.036	1	.871**	0.237	0.199
	Sig.	0.749	0.804	0.863	0.518	0	0	0.005	0.008	0.626	0.362	0.827	0.815		0	0.117	0.191
	N	37	37	37	37	37	36	37	36	45	45	45	45	45	45	45	45
ota3	Pearson	0.18	0.185	-0.05	-0.084	.606**	.562**	0.218	0.279	0.223	0.282	-0.105	0.022	.871**	1	0.199	0.134
	Sig.	0.286	0.273	0.771	0.621	0	0	0.195	0.1	0.141	0.06	0.491	0.887	0		0.19	0.38
	N	37	37	37	37	37	36	37	36	45	45	45	45	45	45	45	45
avpb3	Pearson	-0.202	-0.173	-0.006	0.189	-0.041	0.27	.611**	.658**	0.046	0.034	-0.131	-0.058	0.237	0.199	1	.867**
	Sig.	0.23	0.307	0.971	0.263	0.81	0.111	0	0	0.766	0.827	0.392	0.706	0.117	0.19		0
	N	37	37	37	37	37	36	37	36	45	45	45	45	45	45	45	45
avpa3	Pearson	-0.107	-0.048	0.064	0.084	0.071	.347*	.554**	.539**	0.125	0.12	-0.041	0.092	0.199	0.134	.867**	1
	Sig.	0.53	0.778	0.707	0.619	0.678	0.038	0	0.001	0.412	0.431	0.79	0.547	0.191	0.38	0	
	N	37	37	37	37	37	36	37	36	45	45	45	45	45	45	45	45
** Correlation is significant at the 0.01 level (2-tailed).																	
* Correlation is significant at the 0.05 level (2-tailed).																	

Positive correlations of hormone changes were significant for the relationships between CORT and OT, AVP in the video session. Likewise, CORT was significantly positively correlated with the change in OT during the baby doll session (Table 3).

Table 3. Correlations of hormone level changes. Variables listed as hormone (T, CORT, OT, AVP), before/after (b/a), and session (2=video, 3=baby doll).

		tch2	cortch2	otch2	avpch2	tch3	cortch3	otch3	avpch3
tch2	Pearson	1	-0.185	0.05	-0.148	0.033	-0.172	-0.059	0.263
	Sig.		0.236	0.752	0.349	0.848	0.308	0.727	0.116
	N	43	43	42	42	37	37	37	37
cortch2	Pearson	-0.185	1	.328*	.412**	0.149	0.1	0.182	-0.273
	Sig.	0.236		0.034	0.007	0.38	0.556	0.281	0.102
	N	43	43	42	42	37	37	37	37
otch2	Pearson	0.05	.328*	1	0.047	0.167	-0.018	-0.153	0.062
	Sig.	0.752	0.034		0.768	0.331	0.919	0.373	0.721
	N	42	42	42	42	36	36	36	36
avpch2	Pearson	-0.148	.412**	0.047	1	0.05	-0.195	0.303	-0.145
	Sig.	0.349	0.007	0.768		0.773	0.254	0.073	0.398
	N	42	42	42	42	36	36	36	36
tch3	Pearson	0.033	0.149	0.167	0.05	1	-0.022	-0.141	-0.047
	Sig.	0.848	0.38	0.331	0.773		0.884	0.357	0.758
	N	37	37	36	36	45	45	45	45
cortch3	Pearson	-0.172	0.1	-0.018	-0.195	-0.022	1	.384**	0.242
	Sig.	0.308	0.556	0.919	0.254	0.884		0.009	0.109
	N	37	37	36	36	45	45	45	45
otch3	Pearson	-0.059	0.182	-0.153	0.303	-0.141	.384**	1	-0.13
	Sig.	0.727	0.281	0.373	0.073	0.357	0.009		0.395
	N	37	37	36	36	45	45	45	45
avpch3	Pearson	0.263	-0.273	0.062	-0.145	-0.047	0.242	-0.13	1
	Sig.	0.116	0.102	0.721	0.398	0.758	0.109	0.395	
	N	37	37	36	36	45	45	45	45
* Correlation is significant at the 0.05 level (2-tailed).									
** Correlation is significant at the 0.01 level (2-tailed).									

3.2. Testosterone/Cortisol Ratios

The T/CORT ratio increased significantly during the baby doll session in both the 3-Group ($F_{1,42} = 15.60$, $p < 0.01$, $eta^2 = .27$, Power = .97) and RS-Partner/RS-Parent ($F_{1,41} = 10.97$, $p < 0.01$, $eta^2 = .21$, Power = .90; Table 2) analyses. There was no significant main effect for Group and no significant interaction in the 3-Group analysis. In the RS-Partner/RS-Parent analysis, there was a significant RS-Parent effect ($F_{1,41} = 7.14$, $p = 0.01$ $eta^2 = .15$, Power = .74) with men in the low parent group displaying a significantly higher ratio of T to CORT than men in the high parent group (Table 2). Additionally, there was a significant interaction between RS-Partner and RS-Parent in the baby doll session for T /CORT ratios ($F_{1,41} = 5.36$, $p = 0.03$, $eta^2 = .12$, Power = .62; Figure 2). Men in the low partner/low parent displayed a significantly higher ratio of T to CORT than men in the low partner/high parent ($t_{13} = 3.06$, $p < 0.01$) and men in the high partner/low parent group ($t_{19} = 2.59$, $p = 0.02$). There were no other significant differences in the partner/parent groups. The T/CORT ratio did not differ significantly for any variables in the video session.

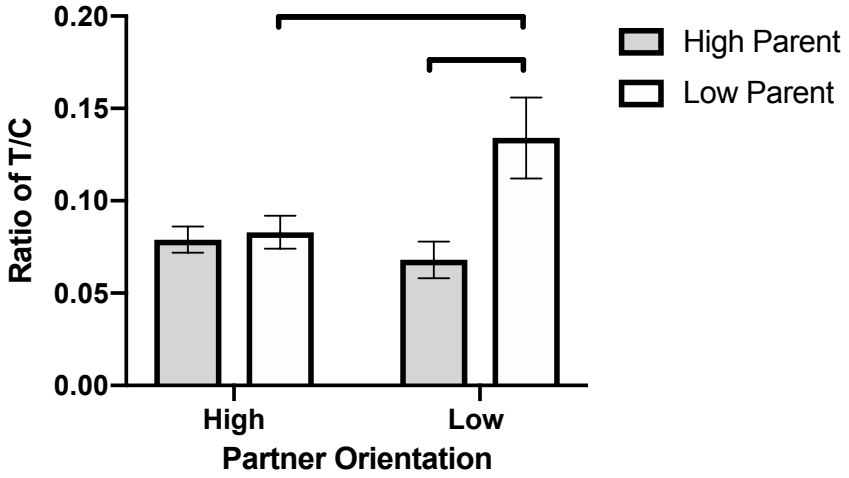

Figure 3. Ratios of T to CORT (nmol/mL; M±SE) in the baby doll session. Men in the low partner/low parent (N = 6) groups had higher ratios of T/C than men in the low partner/high parent (N = 9) and the high partner/low parent (N = 15).

3.3. Oxytocin/Cortisol Ratios

The OT/CORT ratio increased significantly during the baby doll session in the 3-Group ($F_{1,42} = 16.92$, $p < 0.01$, $eta^2 = .25$, Power = .99) and in the RS-Partner/RS-Parent ($F_{1,41} = 22.16$, $p < 0.01$, $eta^2 = .29$, Power = .98; Table 2) analyses. There were no further significant results in the 3-Group model. In the RS-Partner/RS-Parent analysis for the baby doll session, the significant interaction in OT/CORT ratios for session by parent orientation ($F_{1,41} = 4.66$, $p = 0.04$, $eta^2 = .10$, Power = .56) indicated that the increase in the OT/CORT ratio only occurred in the low parent group (Figure 4). There were significant main effects in OT/CORT ratios for both partner ($F_{1,41} = 10.69$, $p < 0.01$, $eta^2 = .21$, Power = .89, low > high) and parent ($F_{1,41} = 16.36$, $p < 0.01$, $eta^2 = .29$, Power = .98, low > high; Table 2, Figure 4). There was also a significant partner/parent interaction ($F_{1,41} = 18.62$, p

< 0.01, $eta^2 = .31$, Power = .99; Figure 4). Post-hoc analyses of the interaction indicated that men in the low partner/low parent group displayed a significantly higher OT/CORT ratio than both men in the low partner/high parent group ($t_{13} = 5.12$, $p < 0.01$) and men in the high partner/low parent group ($t_{19} = 5.26$, $p < 0.01$). There were no differences between the high parent group and the high and low partner groups. There were no significant main effects or interactions in the video session. Taken together, these results suggest that high OT/CORT ratios are associated with the participants least interested in paternal care.

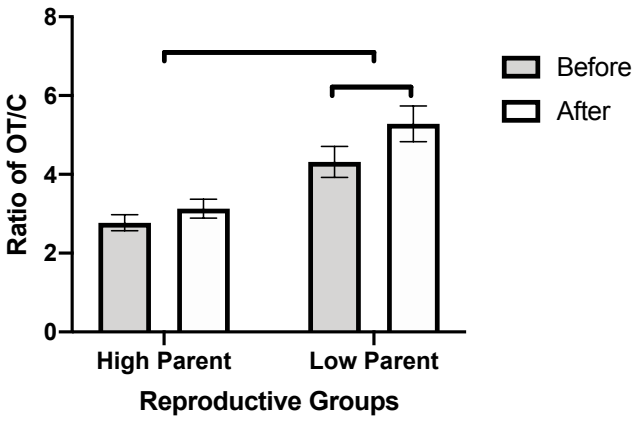

Figure 4. Ratios of OT to CORT (nmol/mL; M±SE) were higher in men in the low parent group (N=15) than in men in the high parent group (N = 30) during the baby doll session. * significant increase in the OT/CORT ratio for men in the low parent group

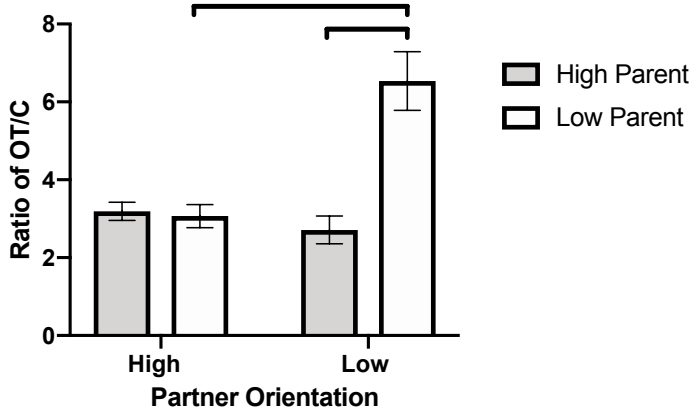

Figure 5. Ratios of OT to CORT (nmol/mL; M±SE) were higher for men in the low partner/low parent (N = 6) than men in the low partner/high parent (N = 9) or high partner/low parent group (N = 15) in the baby doll session.

3.4. Vasopressin/Cortisol Ratios

The AVP/CORT ratio increased significantly during the baby doll session in both the 3-Group ($F_{1,42} = 12.28$, $p < 0.01$) and RS-Partner/RS-Parent analyses ($F_{1,41} = 7.97$, $p < 0.01$, $eta^2 = .16$, Power = .79; Table 2). There was a significant RS-Partner effect with men in the low partner group displaying a higher ratio of AVP/C than men in the high partner group ($F_{1,41} = 4.65$, $p = 0.04$, $eta^2 = .10$, Power = .56). There was also a significant interaction between partnering and parenting strategies in the baby doll session ($F_{1,41} = 6.27$, $p = 0.02$, $eta^2 = .13$, Power = .69). Men in the low partner/low parent groups displayed a higher ratio of AVP/CORT than men in the low partner/high parent group ($t_{19} = 3.23$, $p < 0.01$, Fig. 5) and men in the high partner/low parent groups ($t_{13} = 2.76$, $p = 0.02$; Figure 5). No other combination of groups differed significantly. There were also no significant main effects or interactions for AVP/CORT ratios in the video session.

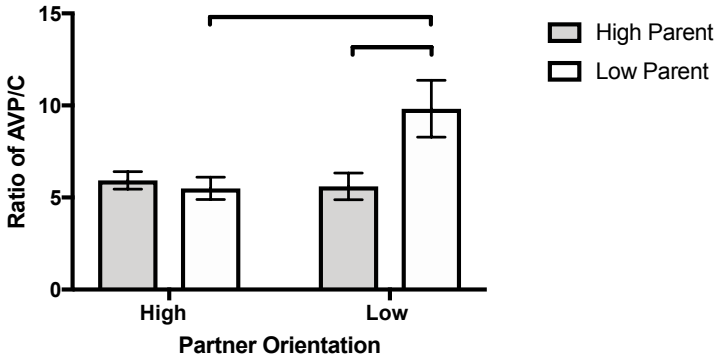

Figure 6. Ratios of AVP to CORT (nmol/mL; M±SE) were higher for men in the low partner/low parent (N = 6) than men in the low partner/high parent (N = 9) or high partner/low parent groups (N = 15).

3.5. Testosterone/Oxytocin Ratios

There was a significant Group effect in the video session of the 3-Group model ($F_{2,39} = 3.91$, $p = 0.03$, $eta^2 = .17$, Power = .67; Table 2) with men in the Bachelor group displaying significantly higher T/OT ratio than men in the Provider or Direct Father groups, however, only the difference between the men in the Bachelor and Direct Father remained significant when Bonferroni test corrected ($p < 0.01$). There were no other significant Group, session, or interaction effects in the 3-Group or RS-Partner/RS-Parent analyses and none in the baby doll analysis ($eta^2 = 0.0$, Power = .05).

3.6. Testosterone/Vasopressin Ratios

There was a significant Group effect in the 3-Group analyses in the video session ($F_{1,39} = 6.16$, $p < 0.01$, $eta^2 = .24$, Power = .87; Table 2), with men in the Bachelor group displaying higher T/AVP ratios than men in the Provider ($p < 0.01$) or Direct Father ($p <$

0.01) groups. Men in the Provider and the Direct Father group were not significantly different from one another ($p = 0.67$). There were no other significant differences in the 3-Group analyses for the video session or in the RS-Partner/RS-Parent analyses for either the baby doll or video sessions (e.g., $eta^2 = .03$, Power = .14).

Table 4. Significant main effects (M±SE, P) in hormone ratio analyses with reference to the figures showing the associated significant interactions. DF – Direct Father, P – Provider, B – Bachelor, LP – Low Parent, HP – High Parent, PR- Partner, PT- Parent

Ratio			3-Group Analysis			RS Partner/RS Parent		
	Session		Main Effects		Interaction	Main Effects		Interaction
		N	Mean ± SE	P		Mean ± SE	P	
T/C								
Before	Baby	45	0.078 ± 0.007	<0.01	-	0.085 ± 0.007	<0.01	-
After		45	0.093 ± 0.007			0.098 ± 0.007		
LP	Baby	15	-	-	-	0.109 ± 0.012	<0.01	Parent x Partner, Fig. 2
HP		30	-	-	-	0.073 ± 0.006		
OT/C								
Before	Baby	45	3.010 ± 0.246	<0.01	-	3.545 ± 0.221	<0.01	Parent x BA, Fig. 3
After		45	3.569 ± 0.290		-	4.207 ± 0.256		
PR	Baby							
High		34	-	-	-	3.129 ± 0.188	<0.01	Parent x Partner, Fig. 4
Low		11	-	-	-	4.624 ± 0.417		
PT	Baby							
High		30	-	-	-	2.951 ± 0.212	<0.01	
Low		15	-	-	-	4.801 ± 0.405		
AVP/C	Baby							
Before		45	5.230 ± 0.429	<0.01	-	6.272 ± 0.425	<0.01	-
After		45	6.245 ± 0.415		-	7.136 ± 0.560		
LP	Baby	11	-	-	-	7.715 ± 0.855	0.04	Parent x Partner, Fig. 6
HP		34	-	-	-	5.594 ± 0.386		
T/OT	Video							
DF		29	0.022 ± 0.002	0.03	-	-	-	-
P		10	0.023 ± 0.003		-	-		
B		3	0.037 ± 0.005		-	-		
T/AVP	Video							
DF		24	0.013 ± 0.001	<0.01	-	-	-	-
P		10	0.014 ± 0.002		-	-		
B		3	0.026 ± 0.004		-	-		

4. Discussion

Hormone levels and ratios differed between sessions and groups, though some of the differences were not in the predicted direction(s). CORT levels decreased significantly more in the baby doll session than in the video session, suggesting that arousal decreased in the parenting but not the partnering context. Men in the low parent group showed a decrease in oxytocin in the baby doll session but there were no changes in T or AVP during either the video or baby doll condition. T/CORT, OT/CORT, and AVP/CORT ratios all increased during the baby doll session and ratios were highest in that session for the least involved men (low partner/low parent group) suggesting that they are the least prone to the higher arousal or greater focus associated with infant care.

4.1. Cortisol (CORT)

CORT decreased more in the baby doll session than in the video session in both model analyses. Providing successful nurturance has been shown to impact male hormone patterns (see van Anders et al., 2012 for a T example) and the decrease in CORT may reflect such an effect. As caregiving scores were high for participants, it is likely that men felt confident in their performance and therefore felt stress relief as the session progressed. It is also possible that the act of providing care was experienced as a positive social engagement and allowed for endogenous OT levels to facilitate decreases in CORT (Heinrichs et al., 2003) or just the simple act of physical contact in a nurturant setting encouraged CORT decrease (Sumioka et al., 2013) which did not happen in the video session with the partner.

4.2. Testosterone (T)

Surprisingly, there were no significant changes in T levels in either session or differences among groups. This finding could be due to a number of factors, including an average of moderate/low baseline T that did not allow for significant change, a lack of emotional or physical saliency in the experimental stimuli (i.e., selected videos and/or baby doll). Previous research indicates that T levels react within the scope of their physiological range, such that men with moderate levels of T, rather than high or low T may have the ability to respond more successfully to partnering and parenting situations. In contrast, other studies have shown that men with higher than average levels of T and lower than average CORT can experience reduced empathy, therefore making it less likely for them to respond to stimuli such as an infant proxy (Zilioli et al., 2015). Additionally, low CORT levels can facilitate T increases in social interactions (Bedgood et al., 2014), high CORT due to pre-session stress may have prohibited T decreases. Lastly, the subjective experience and intent of the participant can also affect the efficacy of the experimental stimuli.

4.3. Oxytocin (OT)

As predicted, the men in the high parent group showed a greater increase in OT levels than the men in the low parent group. These results support studies showing that men with higher OT are more likely to participate in affectionate infant contact (Apter-Levi et al., 2014). Additionally, men with low T respond to affectionate touch with higher OT than men with high T (Gordon et al., 2017). Review of OT studies have shown overall that high OT levels are indicative of men that are socially bonded and more likely

to be paternal (Heinrichs et al., 2009) as well as more sensitive caregivers (Glasper et al., 2019; Rilling, 2013).

4.4. Hormone ratios with CORT in the denominator

All of the hormone ratios with CORT in the denominator increased across the baby doll session, primarily due to decreases in the denominator, CORT. Further, all hormone ratios with CORT in the denominator were higher for the Low Partner/Low Parent group than for each of the comparable groups (Low Partner/High Parent and High Partner/Low parent). While higher ratios were predicted for the least committed men for some ratios (e.g., T/CORT), the results for the other ratios, OT/CORT and AVP/CORT were unexpected.

While all the ratios with CORT in the denominator share the increase during the baby doll session and higher levels in the Low Parent/Low Partner group, the T/C ratio is most consistent with the previous literature. Men in this group would be less likely to be interested in nurturing behaviors as previous research has indicated high T and low CORT to be associated with status seeking/competitive strategies in males (for review see Mehta and Prasad, 2015). Since T levels did not decrease whereas CORT levels did, the simplest explanation for these results is that the group that cared the least about the parental task maintained a high T level but their CORT levels were low due to a lack of stress and/or focus on caring for the doll. Participants in this study did informally report having difficulty thinking of the RealCare baby as being like a real baby. Bos et al. (2018) found that men who reported taking an infant caregiving task more seriously experienced a greater decline in T levels than men that reported taking the task less seriously, which

may explain the overall high T levels also contributing to the high T/C ratio in the less parentally motivated men in this study.

Interpretation of the OT/CORT increase in the baby doll session is even more complex. Although OT levels decreased for men in the low parent group, their OT/CORT ratios increased, indicating that there was a larger decrease in CORT levels than in their OT levels. Men in the low parent/low partner group had the highest OT/CORT ratios and as with the T/CORT ratios, the low levels of CORT suggest a lack of engagement in these men. Studies indicate that OT levels can increase in the presence of positive social interactions or interactions that require positive social engagement, yet remain unchanged in the presence of interactions that either do not require response or in which response will not affect the outcome (Crespi, 2015), as well as situations in which the individual is not motivated to engage.

Results from research on AVP have been complex and often contradictory. Though the AVP/C ratios increased in the baby session in both the 3-Group and RS/RS models, it was men in the low partner/low parent groups that displayed the highest AVP/C ratio. AVP is implicated in precise reactions to sexual and aggressive interactions (van Anders et al., 2011), which may partly explain the higher AVP/C ratios in men in the low partner group. The use of the RealCare baby doll may have introduced a "competition" component to the study rather than a nurturant interaction (for T related example see Eisenegger et al., 2011). Some participants commented that they feel compelled to care for the "baby" at first, but that after a few minutes it felt more like "video game" that they were trying to succeed at. Further investigation of AVP in the partnering and parenting arena is warranted and encouraged. Overall, high ratios of

AVP/C are likely due to decreases in CORT, which were caused by lack of care or concern for the experimental stimuli (i.e., personal perspective or ineffective stimuli).

4.5. Testosterone/Oxytocin and Testosterone/Vasopressin

Men in the Bachelor group of the 3-Group model displayed higher T/OT and T/AVP ratios than other men during the video session. These results are interesting as all other significant differences in ratios were found in the baby doll session. Due to the small number of men in the Bachelor group (N=3), these results are preliminary at best. Analyses conducted in Chapter 1 indicate that men in the Bachelor group had significantly higher levels of T than men in the Provider or Direct Father groups. This elevated T is likely the driver for the higher ratios of T/OT and T/AVP and is consistent with findings that higher T is associated with more interest in sexual rather than parental scenarios. The Steroid/Peptide Theory indicates that when both sexual and parental stimuli are present, sexual stimuli will take priority in the steroid hormone system (i.e., T levels will take priority, van Anders et al., 2011). Further study of men that report both low interest in partnering and parenting should be conducted and include both steroid and neuropeptide hormones to better understand this understudied group.

4.6. Hormone Ratio Method

Hormone ratios have been used in studies of hormones and behavior. However, authors warn that while the hormone ratio method is appealing for its flexibility and ease of use, interpretation can be difficult (Sollberger and Ehlert, 2016). Essentially, hormone ratios allow the variability of the denominator to be removed from the numerator. Other authors have utilized multilevel hierarchical linear modeling, thereby allowing for analyses of baseline and reactive measures of hormones in a step-wise model (Bos et al.,

2018), which may have yielded more refined results when sample sizes are large enough. Future studies should evaluate the distribution of their data and select the most robust statistical method currently available.

5. Conclusions

Results suggest that the suitability of either the 3-Group and RS/RS Models depends on the research question in mind. For example, examination of sexual systems will benefit from use of the 3-Group model as it allows for the broad strokes evaluation of T levels and its associated behaviors. However, the refinement of the RS/RS Model will suit the subtle characteristics of parenting interactions and contexts involving hormones such as OT.

Overall merits of this study include the successful use of the RealCare baby doll as a proxy for infant caregiving, further demonstration of reproductive strategies in human males, and simultaneous examination of multiple hormones and their complex relations. Study weaknesses include the small sample size, possible critiques of the hormone ratio analyses methods, and lack of participants in the Bachelor group. Future studies should prioritize a larger but still broadly demographic sample, retain the multiple experimental contexts, and utilize flexible statistical methods capable of detecting hormone interactions. Results from this study demonstrate the importance of responsive caregiving and the need for further understanding of the CORT response in men/fathers that has implications for the overall well-being of the family unit. These findings illustrate the need for creating supportive environments that encourage healthy development of the father-infant bond.

References

Apter-Levi, Y., Zagoory-Sharon, O., Feldman, R., 2014. Oxytocin and vasopressin support distinct configurations of social synchrony. Brain Res. 1580, 124–132. https://doi.org/10.1016/j.brainres.2013.10.052

Bedgood, D., Boggiano, M.M., Turan, B., 2014. Testosterone and social evaluative stress : The moderating role of basal cortisol. Psychoneuroendocrinology 47, 107–115. https://doi.org/10.1016/j.psyneuen.2014.05.007

Berg, S.J., Wynne-Edwards, K.E., 2001. Changes in testosterone, cortisol, and estradiol levels in men becoming fathers. Mayo Clin. Proc. 76, 582–592. https://doi.org/10.4065/76.6.582

Bos, P.A., Hechler, C., Beijers, R., Shinohara, K., Esposito, G., de Weerth, C., 2018. Prenatal and postnatal cortisol and testosterone are related to parental caregiving quality in fathers, but not in mothers. Psychoneuroendocrinology 97, 94–103. https://doi.org/10.1016/j.psyneuen.2018.07.013

Carré, J.M., Olmstead, N.A., 2015. Social Neuroendocrinology of Human Aggression: Examing the Role of Competition-Induced Testosterone Dynamics. Neuroscience 286, 171–186. https://doi.org/10.1016/j.neuroscience.2014.11.029

Cohen-Bendahan, C.C.C., Beijers, R., van Doornen, L.J.P., de Weerth, C., 2015. Explicit and implicit caregiving interests in expectant fathers: Do endogenous and exogenous oxytocin and vasopressin matter? Infant Behav. Dev. 41, 26–37. https://doi.org/10.1016/j.infbeh.2015.06.007

Crespi, B.J., 2015. Oxytocin, testosterone, and human social cognition. Biol. Rev. 91, 390–408. https://doi.org/10.1111/brv.12175

Dekkers, T.J., van Rentergem, J.A.A., Meijer, B., Popma, A., Wagemaker, E., Huizenga, H.M., 2019. A meta-analytical evaluation of the dual-hormone hypothesis: Does cortisol moderate the relationship between testosterone and status, dominance, risk taking, aggression, and psychopathy? Neurosci. Biobehav. Rev. 96, 250–271. https://doi.org/10.1016/j.neubiorev.2018.12.004

Eisenegger, C., Haushofer, J., Fehr, E., 2011. The role of testosterone in social interaction. Trends Cogn. Sci. 15, 263–271. https://doi.org/10.1016/j.tics.2011.04.008

Erickson, K., Drevets, W., Schulkin, J., 2003. Glucocorticoid regulation of diverse cognitive functions in normal and pathological emotional states. Neurosci. Biobehav. Rev. 27, 233–46. https://doi.org/10.1016/S0149-7634(03)00033-2

Gettler, L.T., Mcdade, T.W., Kuzawa, C.W., 2011. Cortisol and testosterone in Filipino young adult men: Evidence for co-regulation of both hormones by fatherhood and relationship status. Am. J. Hum. Biol. 23, 609–620. https://doi.org/10.1002/ajhb.21187

Gettler, L.T., Ryan, C.P., Eisenberg, D.T.A.A., Rzhetskaya, M., Hayes, M.G., Feranil, A.B., Bechayda, S.A., Kuzawa, C.W., Rzhet-, M., Hayes, M.G., Feranil, A.B., Bechayda, S.A., Kuzawa, C.W., Geoffrey, M., Alan, B., Agustin, S., 2017. The role of testosterone in coordinating male life history strategies: The moderating effects of the androgen receptor CAG repeat polymorphism. Horm. Behav. 87, 164–175. https://doi.org/10.1016/j.yhbeh.2016.10.012

Glasper, E.R., Kenkel, W.M., Bick, J., Rilling, J.K., 2019. More than just mothers: The neurobiological and neuroendocrine underpinnings of allomaternal caregiving. Front. Neuroendocrinol. 53, 100741. https://doi.org/10.1016/j.yfrne.2019.02.005

Gordon, I., Pratt, M., Bergunde, K., Zagoory-Sharon, O., Feldman, R., 2017. Testosterone, oxytocin, and the development of human parental care. Horm. Behav. 93, 184–192. https://doi.org/10.1016/j.yhbeh.2017.05.016

Gordon, I., Zagoory-Sharon, O., Leckman, J.F., Feldman, R., 2010. Oxytocin, cortisol, and triadic family interactions. Physiol. Behav. 101, 679–684. https://doi.org/10.1016/j.physbeh.2010.08.008

Gordon, I., Zagoory-Sharon, O., Schneiderman, I., Leckman, J.F., Weller, A., Feldman, R., 2008. Oxytocin and cortisol in romantically unattached young adults: Associations with bonding and psychological distress. Psychophysiology 45, 349–352. https://doi.org/10.1111/j.1469-8986.2008.00649.x

Heinrichs, M., Baumgartner, T., Kirschbaum, C., Ehlert, U., 2003. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol. Psychiatry 54, 1389–1398. https://doi.org/10.1016/S0006-3223(03)00465-7

Heinrichs, M., von Dawans, B., Domes, G., 2009. Oxytocin, vasopressin, and human social behavior. Front. Neuroendocrinol. 30, 548–557. https://doi.org/10.1016/j.yfrne.2009.05.005

Kelsey, T W, Lucy Q. Li, Rod T. Mitchell, Ashley Whelan, Richard A. Anderson, W.H.B.W., 2014. A validated age-related normative model for male total testosterone shows increasing variance but no decline after age 40 years: Editorial comment. J. Urol. 9, 109346. https://doi.org/10.1016/j.juro.2015.02.062

Kirschbaum, C, Prussner, J, Stone, A, Federenko, I, Gaab, J, Lintz, D, Schommer, N, Hellhammer, D., 1995. Persistent High Cortisol Responses to Repeated Psychological Stress in a Subpopulation of Healthy Men. Psychosom. Med. 57, 468–474.

Kuo, P.X., Braungart-Rieker, J.M., Burke Lefever, J.E., Sarma, M.S., O'Neill, M., Gettler, L.T., 2018. Fathers' cortisol and testosterone in the days around infants' births predict later paternal involvement. Horm. Behav. 106, 28–34. https://doi.org/10.1016/j.yhbeh.2018.08.011

Marazziti, D., Canale, D., 2004. Hormonal changes when falling in love. Psychoneuroendocrinology 29, 931–936. https://doi.org/10.1016/j.psyneuen.2003.08.006

Mehta, P.H., Prasad, S., 2015. The dual-hormone hypothesis: A brief review and future research agenda. Curr. Opin. Behav. Sci. 3, 163–168. https://doi.org/10.1016/j.cobeha.2015.04.008

Miller, S.A., Gallie, D.R., Sleat, D.E., Watts, J.W., Turner, P.C., Wilson, T.M. a, 1988. A Simple Salting Out Procedure for Extracting DNA from Human Nucleated Cells. Nucleic Acids Res. 16, 883–893.

Ponzi, D., Zilioli, S., Mehta, P.H., Maslov, A., Watson, N. V., 2016. Social network centrality and hormones: The interaction of testosterone and cortisol. Psychoneuroendocrinology 68, 6–13. https://doi.org/10.1016/j.psyneuen.2016.02.014

Rilling, J.K., 2013. The neural and hormonal bases of human parentalcare. Neuropsychologia 51, 731–747. https://doi.org/10.1016/j.neuropsychologia.2012.12.017
Romero-Martínez, A., González-Bono, E., Lila, M., Moya-Albiol, L., 2013. Testosterone/cortisol ratio in response to acute stress: A possible marker of risk for marital violence. Soc. Neurosci. 8, 240–247. https://doi.org/10.1080/17470919.2013.772072
Roney, J.R., Simmons, Z.L., Lukaszewski, A.W., 2010. Androgen receptor gene sequence and basal cortisol concentrations predict men's hormonal responses to potential mates. Proc. R. Soc. B Biol. Sci. 277, 57–63. https://doi.org/10.1098/rspb.2009.1538
Smith, A.M., Loving, T.J., Crockett, E.E., Campbell, L., 2009. What's Closeness Got to Do with It? Men's and Women's Cortisol Responses When Providing and Receiving Support. Psychosom. Med. 71, 843–851. https://doi.org/10.1097/PSY.0b013e3181b492e6
Sollberger, S., Ehlert, U., 2016. How to use and interpret hormone ratios. Psychoneuroendocrinology 63, 385–397.
Spanier, G.B., 1976. Measuring Dyadic Adjustment: New Scales for Assessing the Quality of Marriage and Simialr Dyads. J. Marriage Fam.
Storey, A.E., Walsh, C.J., Quiton, R.L., Wynne-Edwards, K.E., 2000. Hormonal correlates of paternal responsiveness in new and expectant fathers. Evol. Hum. Behav. 21, 79–95. https://doi.org/10.1016/S1090-5138(99)00042-2
Sumioka, H., Nakae, A., Kanai, R., Ishiguro, H., 2013. Huggable communication medium decreases cortisol levels. Sci. Rep. 3, 1–6. https://doi.org/10.1038/srep03034
Tabak, B.A., Meyer, M.L., Castle, E., Dutcher, J.M., Michael, R., Han, J.H., Lieberman, M.D., Eisenberger, N.I., 2015. Vasopressin, but not oxytocin, increases empathic concern among individuals who received higher levels of paternal warmth: a randomized controlled trial. Psychoneuroendocrinology 0, 253–261. https://doi.org/10.1016/j.psyneuen.2014.10.006.Vasopressin
Trumble, B.C., Smith, E.A., O'Connor, K.A., Kaplan, H.S., Gurven, M.D., 2013. Successful hunting increases testosterone and cortisol in a subsistence population. Proc. R. Soc. B Biol. Sci. 281. https://doi.org/10.1098/rspb.2013.2876
van Anders, S.M., Goldey, K.L., Kuo, P.X., 2011. The Steroid/Peptide Theory of Social Bonds: Integrating testosterone and peptide responses for classifying social behavioral contexts. Psychoneuroendocrinology 36, 1265–1275. https://doi.org/10.1016/j.psyneuen.2011.06.001
van Anders, S.M., Gray, P.B.P., 2007. Hormones and Human Partnering. Annu. Rev. Sex Res. 18, 60–93. https://doi.org/10.1080/10532528.2007.10559847
van Anders, S.M., Tolman, R.M., Volling, B.L., 2012. Baby cries and nurturance affect testosterone in men. Horm. Behav. 61, 31–36. https://doi.org/10.1016/j.yhbeh.2011.09.012

Walum, H., Westberg, L., Henningsson, S., Neiderhiser, J.M., Reiss, D., Igl, W., Ganiban, J.M., Spotts, E.L., Pedersen, N.L., Eriksson, E., Lichtenstein, P., 2008. Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. Proc. Natl. Acad. Sci. U. S. A. 105, 14153–14156. https://doi.org/10.1073/pnas.0803081105

Wilhelm, K., Parker, G., 1988. The Development of a Measure of Intimate Bonds. Psychol. Med. 18, 225–234.

Zilioli, S., Ponzi, D., Henry, A., Maestripieri, D., 2015. Testosterone, Cortisol and Empathy: Evidence for the Dual-Hormone Hypothesis. Adapt. Hum. Behav. Physiol. 1, 421–433. https://doi.org/10.1007/s40750-014-0017-x

Final Conclusions

by

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Key Points

- First study to combine reproductive behavior, endocrinology, and genetics within the context of human mating and parenting strategies
- Identified two possible models of reproductive strategies, suggesting that partnership may be the gateway for the development of paternal physiology and behavior
- Supported the use of the RealCare baby doll as an infant proxy, allowing for testing of both fathers and non-fathers

1. Research Outcomes

The study goals included the evaluation of the 3-Group model compared to the alternative RS/RS model, further support for use of the infant simulator (RealCare baby), and evaluation of hormone ratios in the context of reproductive strategies in human males. Chapter 1 results supported the hypothesized 3-Group model with men in the Bachelor group reporting demographic data consistent with short-term mating strategies and higher baseline T levels than men in the other two groups. Men in the Provider group exhibited a mix of short-term and long-term mating strategies, whereas men in the Direct Father group generally exhibited long-term mating strategies. Chapter 2 results indicated that OT levels were higher in fathers compared to non-fathers after the video session, men who spent more time with children and men in the Provider group. In contrast to some previous literature (e.g., Feldman et al., 2012), higher OT levels in men in the Provider group were specific to particular recessive homozygous genotypes: *OXTR* 2254298 (GG > AA/AG), *OXTR* 53576 (GG > AA/AG) and *CD38* (CC > AA/AC). Most interestingly,

men in the Provider group experienced an increase in OT levels following the video session with their partner, but a decrease following the baby doll session. Chapter 3 results showed greater decreases in CORT levels during the baby doll session than in the video session. In the baby doll session men in the Low Partner/Low Parent group had higher T/CORT, OT/CORT and AVP/CORT ratios than men in the other groups, suggesting that they were least interested in providing adequate parental care. In addition, T/OT and T/AVP were higher for men in the Bachelor group than men in the Provider or Direct Father groups.

Overall, study results support the use of the 3-Group model when studying partnering and parenting together, specifically when the focus is on T. However, when the study focus is OT in a parenting context, these results suggest using the RS/RS model as it allows for greater distinction between men's partnering and parenting inclinations. As discussed in the Steroid-Peptide Theory (van Anders et al., 2011), subjective experience of partnering and parenting predict either T level increases, especially when sexual behavior is included or T decreases in a nurturing contexts. In the case of OT, social interactions are salient stimuli, but increases can be related to sexual responses, such as those related to orgasm as well as to parenting.

2. Limitations and Relevance

This study was conducted in St. John's, NL, Canada, which is an ideal location for many genetic and cultural studies. However, due to the relatively small community and the invasive nature of the blood collection involved, it was difficult to attract a large number of participants resulting in a small sample. This small sample decreased the power in the analyses and thus minimized the number of significant findings (e.g.,

Providers testosterone levels were not significantly different from Direct Fathers and Bachelors) but in most cases, non-significant findings were associated with small effect sizes, suggesting than even a substantial increase in sample size would not have changed the outcome. Likewise, since most of the participants were recruited through in-person means, there may have been bias in participant selection. Despite these factors, results provide promise in the detection and intervention in cases of insensitive paternal care and abuse. For example, behavioral and hormonal screening of expectant fathers, or men considering parenting, could help identify those men at risk for insensitive care and allow for sensitivity training and counseling prior to fatherhood. Even simply encouraging public awareness and discourse regarding individual variation in relationship and parenting strategies, this research could support individuals as they express their feelings about their potential role in the family and the best ways to support family dynamics and interactions.

Studies have shown that positive family dynamics have significant and long-standing impacts on the adults involved as well as the long-term development of the infants in care, and their future familial relationships. For example, infants raised by sensitive fathers are more likely to become securely attached to their future partners (Lucassen et al., 2011), become more sensitive fathers (Mckay, 2016), and respond to stress with low CORT and higher OT levels (Pierrehumbert et al., 2012). In a study of intranasal OT administration, results indicated that increases in father's OT leads to increases in father-child synchrony as well as infant OT levels (Weisman et al., 2014). Supporting developing families through parenting courses, couples counselling, and early

detection of insensitive family dynamics could mitigate the damaging emotional and health effects of negative family dynamics.

3. Future Directions

Future studies in this area should incorporate sexual history in the evaluation of parenting preferences as sexual satisfaction in the marital relationship is predictive of more stable T levels (Gettler et al., 2013; Puts et al., 2015) and by extension likely increases marital and family stability. In a study of extrapair sexual relationships, results suggest that hormone responses may prioritize sexual stimuli over that of family context (van Anders et al., 2007). These results highlight the need for better understanding of early family development and its complex social interactions.

Building on the personal interactions of the adult partners, there is also the potential impact that the behavior of the infant can have on the parents. Studies of infant soothability have shown that parents exhibit significant hormone changes related to the ease of soothing (Bos et al., 2018; van Anders et al., 2012). In addition to the perception of infant temperament (see Ghera et al., 2006 for an example in mothers), the quality of shared co-parenting has been shown to affect the father's perception of the infant soothability (Burney and Leerkes, 2010). Likewise, the time spent in primary caregiving impacts the behavioral physiology of the caregiver (Gettler et al., 2011). Interestingly, infant OT levels were elevated in infants whose parents rated them as highly soothable (Clark et al., 2013). Use of the RealCare baby can provide the standardization needed to better understand the impact that soothability has on hormones. Studies should also make direct comparisons between a man's own infant and the RealCare baby to evaluate the salience of the RealCare baby stimuli.

An unexpected difficulty of this study was the definition of relationship. While past studies have defined partnered as "committed/co-habitating" (van Anders and Goldey, 2010) or "living together" (Gray and Campbell, 2009), discussions with participants revealed potential complications with such definitions. For example, one study participant discussed extra partner sexual relations that may have affected participant hormone levels. Likewise, another participant discuss the tenuous conditions of his long-term relationship (i.e., potential for future relationship dissolution). Future studies should seek to refine relationship definition and include any possible extra pair interactions. Building on the possibility of relationship variables, future studies would also benefit from assessing sexual frequency and quality to determine any potential impact that sexual interactions could be having on baseline and reactive hormone levels.

Overall, the collection of interactive hormone levels, context specific behavior measures, and hormone-related genotype data will aid in furthering our understanding of the complex system of human partnering and parenting and allow for helpful interventions and support for developing partnerships and families.

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I thank the following individuals for their expertise and assistance throughout all aspects of this study and for their help in writing the manuscript. My unending gratitude to Dr. Anne Storey for help with this paper as well as many other aspects of my professional and personal life. Likewise, I would not be where I am as an academic, or as a person, without the support and guidance of Dr. James Ha. My deepest appreciation to Dr. Carolyn Walsh, Dr. Elizabeth Perry, Dr. Charles Malsbury, Dr. Terry Lynn Young, Dr. Michael Woods, and Dr. Donald McKay for their support in the planning and execution of this study.

References

Bos, P.A., Hechler, C., Beijers, R., Shinohara, K., Esposito, G., de Weerth, C., 2018. Prenatal and postnatal cortisol and testosterone are related to parental caregiving quality in fathers, but not in mothers. Psychoneuroendocrinology 97, 94–103. https://doi.org/10.1016/j.psyneuen.2018.07.013

Burney, R., Leerkes, E., 2010. Links Between Mothers' and Fathers' Perceptions of Infant Temperament and Coparenting. Infant Behav. Dev. 33, 125–135. https://doi.org/10.1016/j.infbeh.2009.12.002

Clark, C.L., John, N.S., Pasca, A.M., Hyde, S.A., Hornbeak, K., Abramova, M., Feldman, H., Parker, K.J., Penn, A.A., 2013. Neonatal CSF Oxytocin Levels are Associated with Parent Report of Infant Soothability and Sociability. Psychoneuroendocrinology 38, 1208–1212. https://doi.org/10.1016/j.psyneuen.2012.10.017

Gettler, L.T., McDade, T.W., Agustin, S.S., Feranil, A.B., Kuzawa, C.W., 2013. Do testosterone declines during the transition to marriage and fatherhood relate to men's sexual behavior? Evidence from the Philippines. Horm. Behav. 64, 755–763. https://doi.org/10.1016/j.yhbeh.2013.08.019

Gettler, L.T., McDade, T.W., Agustin, S.S., Kuzawa, C.W., 2011. Short-term changes in fathers' hormones during father-child play: Impacts of paternal attitudes and experience. Horm. Behav. 60, 599–606. https://doi.org/10.1016/j.yhbeh.2011.08.009

Ghera, M.M., Hane, A.A., Malesa, E.E., Fox, N.A., 2006. The role of infant soothability in the relation between infant negativity and maternal sensitivity. Infant Behav. Dev. 29, 289–293. https://doi.org/10.1016/j.infbeh.2005.09.003

Gray, P.B., Campbell, B., 2009. Human male testosterone, pair bonding, and fatherhood. Endocrinol. Soc. Relationships 15, 119–131.

Lucassen, N., Tharner, A., Van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Volling, B.L., Verhulst, F.C., Lambregtse-Van den Berg, M.P., Tiemeier, H., 2011. The Association Between Paternal Sensitivity and Infant-Father Attachment Security: A Meta-Analysis of Three Decades of Research. J. Fam. Psychol. 25, 986–992. https://doi.org/10.1037/a0025855

Mckay, E.R., 2016. The Impact of Attachment, Parenting Attitudes, and Family History on Emotional, Behavioural, and Physiological Responses to Infant Crying. Doctoral Dissertation. Texas Christian University.

Pierrehumbert, B., Torrisi, R., Ansermet, F., Borghini, A., Halfon, O., 2012. Adult attachment representations predict cortisol and oxytocin responses to stress. Attach. Hum. Dev. 14, 453–476. https://doi.org/10.1080/14616734.2012.706394

Puts, D.A., Pope, L.E., Hill, A.K., Cárdenas, R.A., Welling, L.L.M., Wheatley, J.R., Marc Breedlove, S., 2015. Fulfilling desire: Evidence for negative feedback between men's testosterone, sociosexual psychology, and sexual partner number. Horm. Behav. 70, 14–21. https://doi.org/10.1016/j.yhbeh.2015.01.006

van Anders, S.M., Goldey, K.L., 2010. Testosterone and partnering are linked via relationship status for women and "relationship orientation" for men. Horm. Behav. 58, 820–826. https://doi.org/10.1016/j.yhbeh.2010.08.005

van Anders, S.M., Goldey, K.L., Kuo, P.X., 2011. The Steroid/Peptide Theory of Social Bonds: Integrating testosterone and peptide responses for classifying social behavioral contexts. Psychoneuroendocrinology 36, 1265–1275. https://doi.org/10.1016/j.psyneuen.2011.06.001

van Anders, S.M., Hamilton, L.D., Watson, N. V., 2007. Multiple partners are associated with higher testosterone in North American men and women. Horm. Behav. 51, 454–459. https://doi.org/10.1016/j.yhbeh.2007.01.002

van Anders, S.M., Tolman, R.M., Volling, B.L., 2012. Baby cries and nurturance affect testosterone in men. Horm. Behav. 61, 31–36. https://doi.org/10.1016/j.yhbeh.2011.09.012

Weisman, O., Zagoory-Sharon, O., Feldman, R., 2014. Oxytocin administration, salivary testosterone, and father–infant social behavior Omri. Prog. Neuropsychopharmacol. Biol. Psychiatry 49, 47–52. https://doi.org/10.1016/j.pnpbp.2013.11.006

Health Research Ethics Authority

Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

September 3, 2013

Ms. Hayley Alloway
Department of Psychology
Memorial University of Newfoundland

Dear Ms. Alloway

Reference #13.105

RE: Steroid-Peptide Interaction in Human Paternal Behavior and Pair-bonding

This will acknowledge receipt of your correspondence.

This correspondence has been reviewed by the Chair under the direction of the Board. ***Full board approval*** of this research study is granted for one year effective **August 22, 2013.**

This is to confirm that the Health Research Ethics Board reviewed and approved or acknowledged the following documents (as indicated):

- Application, approved
- Revised Demographic Data, approved
- Revised consent form, dated August 29, 2013, approved

MARK THE DATE

This approval will lapse on **August 22, 2014**. It is your responsibility to ensure that the Ethics Renewal form is forwarded to the HREB office prior to the renewal date. *The information provided in this form must be **current to the time of submission** and submitted to HREB **not less than 30 nor more than 45 days** of the anniversary of your approval date.* The Ethics Renewal form can be downloaded from the HREB website http://www.hrea.ca.

The Health Research Ethics Board advises THAT IF YOU DO NOT return the completed Ethics Renewal form prior to date of renewal:

- *Your ethics approval will lapse*

email: info@hrea.ca Phone: 777-8949 FAX: 777-8776

- *You will be required to stop research activity immediately*
- *You may not be permitted to restart the study until you reapply for and receive approval to undertake the study again*

Lapse in ethics approval may result in interruption or termination of funding

It is **your responsibility to seek the necessary approval from the Regional Health Authority or other organization as appropriate.**

Modifications of the protocol/consent are not permitted without prior approval from the Health Research Ethics Board. Implementing changes in the protocol/consent without HREB approval may result in the approval of your research study being revoked, necessitating cessation of all related research activity. Request for modification to the protocol/consent must be outlined on an amendment form (available on the HREB website) and submitted to the HREB for review.

This research ethics board (the HREB) has reviewed and approved the research protocol and documentation as noted above for the study which is to be conducted by you as the qualified investigator named above at the specified site. This approval and the views of this Research Ethics Board have been documented in writing. In addition, please be advised that the Health Research Ethics Board currently operates according to *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans*; *ICH Guidance E6: Good Clinical Practice* and applicable laws and regulations. The membership of this research ethics board is constituted in compliance with the membership requirements for research ethics boards as defined by *Health Canada Food and Drug Regulations Division 5; Part C.*

Notwithstanding the approval of the HREB, the primary responsibility for the ethical conduct of the investigation remains with you.

We wish you every success with your study.

Sincerely,

Dr. Fern Brunger
Chair, Non-Clinical Trials
Health Research Ethics Board

C C VP Research c/o Office of Research, MUN
VP Research c/o Patient Research Centre, Eastern Health
HREB meeting date: September 5, 2013

email: info@hrea.ca Phone: 777-8949 FAX: 777-8776

Are you a **20-45 year old man?**

Would you be willing to participate in a study of **hormones** and **genetics**?

The study session takes about one to two hours to complete. To take part, you will come to Memorial University, where we will take a blood sample (from your arm with a needle), ask you to interact with a life-like baby doll or your own infant for 25 minutes, take another blood sample, and fill-out questionnaires about your childhood, relationships, and experience with children. During a second session, you will come in and watch a short movie clip (alone, or with your spouse), with blood samples before and after. Though the study may not benefit you directly, you will be contributing to further understanding of family dynamics. For further information about participating in the study, please call or email. We look forward to hearing from you soon!

Contact: **Hayley Alloway, Investigator**

709-769-0898, **h.alloway@mun.ca**

Cognitive and Behavioral Ecology Programme

Memorial University

Department of Psychology
St. John's, NL. Canada A1B 3X9
Tel.: 709 864-7665, Fax: 709 864 2430

Consent to Take Part in Research

TITLE: Steroid-Peptide Interaction in Human Paternal Behavior and Pair-bonding

INVESTIGATOR(S): Hayley Alloway & Dr. Anne Storey

You have been invited to take part in a research study. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. You can decide not to take part in the study. If you decide to take part, you are free to leave at any time.

Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.

Please read this carefully. Take as much time as you like. If you like, take it home to think about for a while. Mark anything you do not understand, or want explained better. After you have read it, please ask questions about anything that is not clear.

The researchers will:

- discuss the study with you
- answer your questions
- keep confidential any information which could identify you personally
- be available during the study to deal with problems and answer questions

1. Introduction/Background:

Research has shown that the hormone levels of men change in the beginning of a new relationship and when they become parents. Some of these studies have found different results, especially in the dad's behavior. Those differences could be due to the relationship between the man and his partner, or his interaction with the infant. To understand this better, we will measure the hormone levels of men in different situations and compare them to their behavior and genetics.

2. Purpose of study:

This study will help us understand what makes a man decide to have a long-term relationship and become a dad.

3. Description of the study procedures:

1. You will visit our laboratory, two times (about 1 week apart), where we will take two blood samples. We will take the samples approximately 30 minutes apart.

2. During the 30 minutes between the blood samples, you will participate in an infant interaction (your baby/RealCare doll) and a romantic interaction (watch a movie clip alone/with your spouse). During the infant interaction you interact with the doll/your baby for 25 minutes. During the visit that you interact with your spouse, you will watch a romantic video for approximately 25 minutes. The video will depict the development of a romantic relationship. It will not include any nudity or sexual behavior.

3. You will complete a series of questionnaires about your personal and childcare related experiences.

4. Length of time:

Each session will last approximately 1-2 hours.

5. Possible risks and discomforts:

Some people experience slight bruising at the blood collection site. In rare cases, an infection at the collection site may occur. If an infection should occur, please contact your primary physician.

6. Benefits:

It is unknown whether this study will benefit you.

7. Liability statement:

Signing this form gives us your consent to be in this study. It tells us that you understand the information about the research study. When you sign this form, you do not give up your legal rights. Researchers or agencies involved in this research study still have their legal and professional responsibilities.

8. What about my privacy and confidentiality?

Protecting your privacy is an important part of this study. Every effort to protect your privacy will be made, however it cannot be guaranteed. For example, we may be required by law to allow access to research records.

When you sign this consent form you give us permission to

- Collect information from you
- Share information with the people conducting the study
- Share information with the people responsible for protecting your safety

Access to records

The members of the research team will **NOT** see study records that identify you by name.

Use of your study information

The research team will collect and use only the information they need for this research study.

This information will include your

- date of birth
- sex
- family history
- medical conditions
- medications
- information from study sessions and questionnaires

Your name and contact information will be kept secure by the research team in Newfoundland and Labrador. Your contact information will not be shared with others without your permission. Your name will not appear in any report or article published as a result of this study.

Information collected for this study will kept for five years.

If you decide to withdraw from the study, the information collected up to that time will be destroyed. This information will only be used for the purposes of this study.

Information collected and used by the research team will be stored in the Psychology Department of Memorial University. Hayley Alloway and Anne Storey are the people responsible for keeping it secure.

Your access to records

You may ask Hayley Alloway or Dr. Anne Storey to see the information that has been collected about you.

9. Questions or problems:

If you have any questions about taking part in this study, you can meet with the investigator who is in charge of the study at this institution. That person is:

Principal Investigator's Name and Phone Number

Hayley Alloway, 709-769-0898

Or you can talk to someone who is not involved with the study at all, but can advise you on your rights as a participant in a research study. This person can be reached through:

Ethics Office
Health Research Ethics Authority
709-777-6974 or by email at info@hrea.ca

After signing this consent you will be given a copy.

Signature Page

Study title: Steroid-Peptide Interaction in Human Paternal Behavior and Pair-bonding

Name of Principal Investigator: Hayley Alloway, Ph.D. Candidate

To be filled-out and signed by the participant:

Please check as appropriate:

I have read the consent. Yes { } No { }
I have had the opportunity to ask questions/to discuss this study. Yes { } No { }
I have received satisfactory answers to all of my questions. Yes { } No { }

I have received enough information about the study. Yes { } No { }
I have spoken to Hayley Alloway and he/she has answered my questions Yes { } No { }
I understand that I am free to withdraw from the study Yes { } No { }

- at any time
- without having to give a reason

I understand that it is my choice to be in the study and that I may not benefit Yes { } No { }
I understand how my privacy is protected and my records kept confidential Yes { } No { }
I agree to take part in this study. Yes { } No { }

_____ _____ _____
Signature of participant Name printed Year Month Day

To be signed by the investigator or person obtaining consent

I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in the study, any potential risks of the study and that he or she has freely chosen to be in the study.

_____ _____ _____
Signature of investigator Name printed Year Month Day

Telephone number: _____

Demographic Data – Please answer all questions to the best of your ability. For any question that you may not be able to answer at this time, you are encouraged to contact the research team with any information that you discover later. If there are any questions that you are not comfortable answering, please leave them blank. This questionnaire is completely CONFIDENTIAL.

1. Date of Birth: _____

2. Occupation: _____Years in occupation: _____

3. Place of Birth (Country and Province/State): _____

Health

4. Weight: _____ Height: _____

5. On average, how many hours of sleep do you get each day?_____

6. Have you participated in vigorous activity today?_____

What type?_____For how long?_____

7. Do you smoke cigarettes? _____How many per day?_____

8. Do you drink alcohol? _____How many drinks on average per week? _____

9. Are you currently taking any medication(s)?_____

If so, please list (especially medications that include hormones such as cortisol).

_____

10. How stressful do you expect the blood collection to be?

Not stressful Moderately stressful Very stressful

Family History

11. Were you breast- or bottle-fed?_____For how long?_____

12. Was your mother's labor induced when you were born? _____

13. How many siblings do you have? _____

14. How old were you when your siblings were born?_____

15. As a child, did you spend much time caring for younger siblings/relatives?_____

Experience with Children

16. How much time do you currently spend with children (i.e. your own, nieces, friend's children, etc)?_____

17. What type of activities do you participate in with young children (i.e. childcare, sporting events, etc)?_____

18. Do you have children? _____

If you have children, please go to the next section.

If you do not have children, would you like to?_____

How many?_____

(Complete the following if you have children)

19. How many children do you have?_____Ages?_____

20. How many hours do you typically spend caring for your child per day?_____

21. What do you like to do with your children?_____

22. Please describe your feelings about being a father._____

_____

Relationship History

23. Relationship Status: Single Dating Living Together

Married Separated Divorced

Are you satisfied with your relationship status?_____

24. If you are in a relationship, how long have you been together?_____

25. Please describe your feelings about being in a long-term, committed relationship._____

_____

Sexual History

26. Are you currently involved in a consistent sexual relationship?_____

27. On average, how often are you having sex per week?_____

Are you satisfied with your current sexual relationship? _____

28. How many sexual partners have you had in total?_____

29. How many sexual partners have you had in the past year?_____

30. How many sexual partners do you expect to have in the future?_____

31. Sexual orientation:_____

DYADIC ADJUSTMENT SCALE

Most persons have disagreements in their relationships. Please indicate below the approximate extent of agreement or disagreement between you and your partner for each item on the following list.

	Always Agree	Almost Always Agree	Occa-sionally Disagree	Fre-quently Disagree	Almost Always Disagree	Always Disagree
1. Handling family finances	5	4	3	2	1	0
2. Matters of recreation	5	4	3	2	1	0
3. Religious matters	5	4	3	2	1	0
4. Demonstrations of affection	5	4	3	2	1	0
5. Friends	5	4	3	2	1	0
6. Sex relations	5	4	3	2	1	0
7. Conventionality (correct or proper behavior)	5	4	3	2	1	0
8. Philosophy of life	5	4	3	2	1	0
9. Ways of dealing with parents or in-laws	5	4	3	2	1	0
10. Aims, goals, and things believed important	5	4	3	2	1	0
11. Amount of time spent together	5	4	3	2	1	0
12. Making major decisions	5	4	3	2	1	0
13. Household tasks	5	4	3	2	1	0
14. Leisure time interests and activities	5	4	3	2	1	0
15. Career decisions	5	4	3	2	1	0
	All the time	Most of the time	More often than not	Occa-sionally	Rarely	Never
16. How often do you discuss or have you considered divorce, separation, or terminating your relationship?	0	1	2	3	4	5
17. How often do you or your mate leave the house after a fight?	0	1	2	3	4	5
18. In general, how often do you think that things between you and your partner are going well?	5	4	3	2	1	0
19. Do you confide in your mate?	5	4	3	2	1	0
20. Do you ever regret that you married? (*or lived together*)	0	1	2	3	4	5
21. How often do you and your partner quarrel?	0	1	2	3	4	5
22. How often do you and your mate "get on each other's nerves?"	0	1	2	3	4	5

	Every Day	Almost Every Day	Occa-sionally	Rarely	Never
23. Do you kiss your mate?	4	3	2	1	0
	All of them	Most of them	Some of them	Very few of them	None of them
24. Do you and your mate engage in outside interests together?	4	3	2	1	0

How often would you say the following events occur between you and your mate?

	Never	Less than once a month	Once or twice a month	Once or twice a week	Once a day	More often
25. Have a stimulating exchange of ideas	0	1	2	3	4	5
26. Laugh together	0	1	2	3	4	5
27. Calmly discuss something	0	1	2	3	4	5
28. Work together on a project	0	1	2	3	4	5

These are some things about which couples sometimes agree and sometime disagree. Indicate if either item below caused differences of opinions or were problems in your relationship during the past few weeks. (Check yes or no)

	Yes	No	
29.	0	1	Being too tired for sex.
30.	0	1	Not showing love.

31. The dots on the following line represent different degrees of happiness in your relationship. The middle point, "happy," represents the degree of happiness of most relationships. Please circle the dot which best describes the degree of happiness, all things considered, of your relationship.

0	1	2	3	4	5	6
Extremely Unhappy	Fairly Unhappy	A Little Unhappy	Happy	Very Happy	Extremely Happy	Perfect

32. Which of the following statements best describes how you feel about the future of your relationship?

5 I want desperately for my relationship to succeed, and *would go to almost any length* to see that it does.

4 I want very much for my relationship to succeed, and *will do all I can* to see that it does.

3 I want very much for my relationship to succeed, and *will do my fair share* to see that it does.

2 It would be nice if my relationship succeeded, but *I can't do much more than I am doing* now to help it succeed.

1 It would be nice if it succeeded, but I *refuse to do any more than I am doing* now to keep the relationship going.

0 My relationship can never succeed, and *there is no more that I can do* to keep the relationship going.

INTIMATE BOND MEASURE

The Intimate Bond Measure (IBM) was developed to measure the dimensions of care and control between partners in an intimate relationship. It is a measure where partners can rate the other.

Scoring Protocol

- The Intimate Bond Measure consists of 24 items with 2 subscales: 12 items for the care dimension, and 12 for the control dimension.
- All items have equivalent likert scaling from 0 to 3 (4 options).
- Higher scores on the dimensions indicate higher perceived care and control.
- Both subscales have a minimum score of 0 and a maximum score of 36.

Total Scores:
Care (clear) = Total of all clear (unshaded) scores (12 items)
Control (shaded) = Total of all shaded scores (12 items)

Reference:
Wilhelm, K., Parker, G. (1988) The development of a measure of intimate bonds.

This questionnaire lists some attitudes and behaviours which people reveal in their close relationships.
Please judge your partner's attitudes and behaviour towards you in recent times and tick the most appropriate box for each item.

	TRUE	Moderately	Somewhat	Not At All
1. Is very considerate of me				
2. Wants me to take his/her side in an argument				
3. Wants to know exactly what I'm doing and where I am				
4. Is a good companion				
5. Is affectionate to me				
6. Is clearly hurt if I don't accept his/her views				
7. Tends to try to change me				
8. Confides closely in me				
9. Tends to criticise me over small issues				
10. Tends to order me about				
11. Insists that I do exactly as I'm told				
12. Is physically gentle and considerate				
13. Makes me feel needed				
14. Wants me to change in small ways				
15. Is very loving to me				
16. Seeks to dominate me				
17. Is fun				
18. Wants to change me in big ways				
19. Tends to control everything I do				
20. Shows his/her appreciation of everything I do				
21. Is critical of me in private				
22. Is gentle and kind to me				
23. Speaks to me in a warm and friendly voice				
24. Understands my problems and worries				

Post-Interaction Questionnaire (Video)

Please answer the following questions to the best of your ability. Skip those questions that you feel unable or unwilling to answer.

1. Did you enjoy watching the videos ? Yes No

    a. Describe your experience of watching the videos.

    _____

    _____

2. Which of the videos did you prefer? Signs (1st) The Notebook clip (2nd)

3. Did you find the interaction stressful? Yes No

    a. If yes, how stressful? 1 2 3 4 5

    Not at all Very Stressful

4. Did you find the blood collection stressful? Yes No

    a. If yes, how stressful? 1 2 3 4 5

    Not at all Very Stressful

Post-Interaction Questionnaire (RealCare Baby)

Please answer the following questions to the best of your ability. Skip those questions that you feel unable or unwilling to answer.

5. Did you enjoy caring for the baby doll? Yes No

    a. Describe your experience of caring for the baby doll.

    _____

    _____

6. Did you find the interaction with the baby doll stressful? Yes No

    a. If yes, how stressful? 1 2 3 4 5
    Not at all Very Stressful

7. Did you find the doll similar to a real baby? Yes No

    a. If not, what was different about the doll?

    _____

8. Did you find the blood collection stressful? Yes No

    a. If yes, how stressful? 1 2 3 4 5
    Not at all Very Stressful