THE UNIVERSITY OF CHICAGO

THE ONTOGENY OF SEXUAL MATURATION:
FACTORS AFFECTING THE DEVELOPMENT OF SEXUAL SIGNAL PRODUCTION, PERCEPTION, AND BEHAVIOR IN ADOLESCENT FEMALE RHESUS MACAQUES

A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE SOCIAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF COMPARATIVE HUMAN DEVELOPMENT

BY
SEAN PATRICK COYNE

CHICAGO, ILLINOIS
JUNE 2017

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

There are a great multitude of people I must thank who have been critical to my success in both my Ph.D. and in my life. First and foremost, I must thank my adviser, Dario Maestripieri, for accepting me into his lab as a young master's student fresh out of undergrad and giving me the opportunity to work in the field with primates. Hiring me as his lab manager, and accepting me as his Ph.D. student provided me with endless opportunities for success, and his support in all my endeavors always goes above and beyond anything a student could hope for in an adviser. I am also grateful to my committee members, Martha McClintock and Greg Norman, for their support and feedback on my proposal and dissertation, which were instrumental in my success.

Thank you to the many people who assisted me on this project in the field in so many capacities: Auberi Courchay, Alex Georgiev, Jesus Madrid, Paola Rullan, Greg Ruber, Michelle Evans, Gabe Stump, Stacy McCarthy, Amaury Michele, and Aneila Hogan. Additionally, I must thank my three personal field assistants in particular: Alec Luro, Clara Dell, and Aparna Chandrashekar; the three of you were my roommates, assistants, and most importantly friends, without any of you this project would not have been possible. Finally, Christine Fleener, no amount of words can describe how necessary you were to my success as we conducted our fieldwork together, but I know you know how much your help and support meant to me. On the technical side of things, I must also extend a huge thank you to John Addicott for creating the software used throughout my data collection and processing. Additionally, a big thank you must go to Shreya Jaiswal for joining the lab and willingly becoming a second coder!

Remote assistance from Chicago was also critical to my success, and I would be remiss if I didn't also thank my lab mates on the human side of the lab. James Marvel-Coen, you have been a great friend and supporter during my write up period, keeping me calm and level when I need it. Nora Nickels and Andrea Henry, there is absolutely no way I would have been able to complete this program without the two of you. Your laughter, tears, car rides, hugs, dance parties, and lunch breaks mean more to me that words will ever be able to say.

There are three people who I credit with making me such a success as a field researcher. First, Constance Dubuc, you have been such a huge supporter of mine over so many field seasons, helping me plan, execute, analyze and interpret all my projects; I hope we continue to collaborate for years to come. Second, James Higham, you have helped me in so many capacities from project planning, serving as a reference, training me on field methods, teaching me physiological methods and providing the lab space to do so, and also served as my outside reader for my proposal. Thank you, truly, for all you've done for me, without which I would never have succeeded in any of my projects. Finally, Tara Mandalaywala, you brought me to my first field season, and supported me in person or remotely for every other one since. I truly would still be lost on Cayo without you. Our personal friendship and professional collaboration are more important to me than any other, and know that you are the one of the biggest components to my continued success.

My field work would not have been possible without the support from the wonderful people at the Caribbean Primate Research Center. I must extend a huge thank you to Angie Ruiz-Lambides, Nahiri Rivera-Barreto, Giselle Caraballo-Cruz, Edgar Davila, Julio Resto and Janet Rivera. Additionally, thank you to the wonderful boatman getting us to and from the field: Omar, Miguel, Danny, Josue, and William.

A special thank you must also go to Michael and Andrea Heistermann at the German Primate Center. Their kindness and patience in training me to perform physiological assays was what made my time in Germany so wonderful. The openness and welcoming nature of your lab made me feel instantly at ease when living in a country where I didn’t speak the language or know anyone else!

I am thankful for the funding I received from the Rynerson Fellowship, the Hinds Fund Research Grant, and the Social Sciences Division Long Term Project Grant. I am also grateful for the support and funding from the Department of Comparative Human Development and the Institute for Mind and Biology. A special thank you must be extended for the administrative assistance from Janie Lardner and Spencer Bonadeo in Comparative Human Development, Mike McCarthy, Janet Capodagli, Scott Bradwell, Sharon Ligue, and James Licklider from the IMB, and Brett Baker in the Dean’s office.

Clearly, many people have been instrumental in my success, but there are four more people I must thank in particular. My mother, father, and sister have always been my biggest supporters and advocates, even if they never fully understood exactly what my research was about! You three have always been just a phone call away, and willing to hop on a plane when I needed it, through all the hardships that I’ve faced in this journey. I love you all so very much, thank you for helping me achieve my dream. Finally, to my partner, Bryan, I thank you for being there for me in this difficult journey. You have never been anything but my biggest advocate, encouraging me to stay positive and keep going when I had doubts. I know I would not have been able to finish my doctorate without your support.

ABSTRACT

Despite the fact that variation in age of first reproduction is one of the most important life history characteristics determining lifetime reproductive success, little work has been done describing the interindividual variation in this trait. While some work has demonstrated the important of heritable factors affecting age at first reproduction, such as dominance rank and genetic heritability, the majority of the variation comes from as yet unexplained environmental influences. In this dissertation, I examine several environmental factors that may affect variation in the age of first reproduction in adolescent female rhesus macaques from a single birth cohort on Cayo Santiago, Puerto Rico. Chapter 2 demonstrates that dominance rank is important for determining age at first reproduction, and that this may in fact be related to differences in body condition. These body condition differences are likely the cause for variation in latency to begin cycling in the mating season, which was an important factor in determining whether or not a female became pregnant. Chapter 3 examines the changing physiology and social behavior between the juvenile and adolescent period by examining the changes in stress physiology and behavior, as well as grooming behavior. Results demonstrate, as predicted, that stress levels do increase during puberty as do grooming rates with other adult females. Chapter 4 explores the function of a sexual signal present only in adolescent females, the hindquarter swelling. However, the swelling does not seem to fit into either of the two existing models of explanation for this trait, and may in fact be a non-functional side effect of reacting to newly circulating female sex hormones. Finally, Chapter 5 explores the development of a perceptual bias for preferentially looking at darker red male faces. Together, these studies provide a comprehensive look at the changing physiology and behavior of adolescents as they enter adulthood, and begin to explain variation in the important life history trait of age at first reproduction.

Chapter 1

Introduction

Life history theory is the conceptual tool long used by biologists to understand and explain the differential allocation of energetic resources by organisms as they move through various stages in the life cycle. Life history theory simply states that because organisms have a limited set of resources available, they must optimize the “spending” of these resources on different activities such as growth, reproduction, and somatic maintenance (Stearns 1992). Life history traits vary widely, and can be used to explain how evolutionary pressures have shaped both the differences between different species, as well as different individuals within a single species.

Puberty is one of the key developmental stages in the lives of many animals, including humans. Puberty is generally considered the transition period during which an individual becomes a reproductively active adult, and age at first reproduction (AFR) is perhaps the biggest determinant of lifetime reproductive success (Stearns 1976). While much research has focused on the reproductive strategies of adults, comparatively little is known about these earlier stages of sexual maturation (i.e. puberty) and how they determine the onset of an individual’s reproductive career. AFR can be affected by a variety of factors including parental rank, seasonal effects, birth order, and heritability (e.g., in rhesus monkeys, Blomquist 2012). Traditionally, much of the existing research about puberty focuses largely on understanding the proximate mechanisms that predict when puberty will begin, but little else (e.g., McClintock and Herdt 1996). Because puberty is accompanied by a vast suite of changes across many characteristics (e.g., physiology, social behavior, neuroanatomy), there are still many aspects of the maturation process we still do not fully understand. Additionally, studying puberty in humans presents a set of additional challenges. First, humans have one of the longest juvenile development periods in the animal kingdom (Bjorkland et al. 2005). Additionally, there are a variety of sociocultural factors that affect the timing of sexual maturity in humans (Ellis 2004) or even how to define puberty (Crockett 1997). Animal models of the sexual maturation process often prove extremely profitable due their increased growth rate relative to humans. Non-human primates are one of the best models for understanding this critical life stage because they share so many characteristics with humans including genetics, neuroanatomy, neurochemical and neuroendocrine function, cognition, and social behavior (reviewed in Phillips et al. 2014), and their progression through life history stages mirror those of humans, though vary widely in their timing (Leigh and Blomquist 2011) setting up ideal studies for the study of evolutionary influences on life history traits.

The transition into sexual maturity is such an important event for a variety of reasons: it is energetically costly, the process is irreversible, and it is a large determinant of lifetime reproductive success. There is an inevitable tradeoff between current and future reproductive opportunities, and as such, animals must balance the energetic and developmental costs in order to optimally time maturation. For example, in many primate species (including humans), there is a hormonal cascade set off by the Gonadotropin-releasing hormone (GnRH), which signals to the body that it is time to start the process of sexual maturity. The timing of its release, however, is variable and seems to be signaled both by food availability and body condition (Foster and Nagatani 1999). The onset of puberty signals a reduction, if not complete termination, of somatic body growth in order to invest in reproductive growth. This tradeoff can be extremely problematic, therefore, if it occurs at an inappropriate time, since undersized animals are often less fecund than larger or more fully grown individuals (Borries et al. 2011). There are however, many factors that can help influence the “correct” time to set off this cascade of changes. Early life experiences can be especially influential in an animal’s development as they typically indicate likely future environmental conditions. If an animal’s future conditions are likely to be extremely hostile or unpredictable, it may be evolutionary advantageous to fast-track its sexual maturity to attempt sub-optimal reproduction rather than waiting for a species-typical “optimal” timing and risk never reproducing.

One of the biggest early life influences in many mammals is parental, particularly maternal, treatment. For example, in primates early experiences of stress via maternal treatment (e.g., protectiveness, rejection, or abuse) is hugely influential on stress resilience and vulnerability later in life (reviewed in Parker and Maestripieri 2011). This is particularly important, as we know from human literature that children raised in abusive homes are more likely to develop internalizing psychological disorders (Turner et al. 2011), attempt and commit suicide (Dube et al. 2001), and use illicit drugs (Dube et al. 2006). However, there are potentially many psycho-social factors that may affect these outcomes from being raised in an abusive home, and therefore it is important to model the effects of early life experiences across a naturally occurring variation in early life stress to understand how these experiences affect sexual maturity.

Non-human primates (NHPs) have been the focus of much research looking at maternal influences on offspring development and behavior. For example, NHP maternal styles can be broken down across two different continuums: 1) rejecting/accepting, and 2) controlling/laissez-faire (Fairbanks 1996). Across these continuums, mothers often act in consistent, predictable ways towards their infants, such as restraining the infant to prevent it from leaving maternal proximity. These maternal behaviors often exert strong behavioral and physiological responses in infants, such as increased number of tantrums and increased basal cortisol levels (Maestripieri 2002, Mandalaywala et al. 2014). Further, a female's maternal style is often similar to her mother's which may have both learned and genetic influences (Maestripieri et al. 2007, Maestripieri et al. 2005). A particular sphere of maternal influence which factors critically in offspring development, and has been well documented in primates, is experiences of stress. In humans, extreme amounts of stress early in life can lead to increased risk for the development of mood disorders such as anxiety and depression (Heim and Nemeroff 2001). Originally the underlying assumption was that as early life stress experiences increased, an individual's risk for these mood disorders increased respectively. However, more recent work has demonstrated a more complex picture leading credence to a model dubbed "stress inoculation." Lyons and Parker (2007) have demonstrated that a moderate amount of early life stress is a good thing for coping with stress, as "inoculated" monkeys are better at reacting to novel stressors in the environment than are those who receive too mild or too much early life stress.

While the above study on stress experiences were completely controlled under artificial laboratory settings, it can be applied to the wide variation of stressful experiences that naturally occur across populations of NHPs. A particularly salient case of maternal induced early life adversity comes from maternal abuse and neglect. These behaviors are common in across many primate species, both in captivity and under more natural conditions. For example, Maestripieri et al. (2006) demonstrated that infants who were rejected often early in life have lower CSF serotonin metabolites (5-HIAA) than infants who were not rejected. As serotonin is important for emotional regulation, these decreased levels seem to indicate a possible mechanism for the intergenerational transmission of abusive and neglectful behaviors seen in females. In addition to influencing emotional regulation and monoaminergic function, early life stress experiences also influence hypothalamic-pituitary-adrenal (HPA) axis development. Along with the response of the autonomic nervous system, the HPA axis coordinates the maintenance of homeostasis in the presence of a challenge or "stressor." The HPA axis enables an individual to mobilize energetic resources where needed and conserving those that are not needed, and both the HPA axis and stress regulation are heavily influenced by early life experiences. For example, Sanchez and colleagues (2010) investigated the development of the HPA axis in abused and non-abused rhesus macaques, finding that individuals with high amounts of early life stress have hyperreactive HPA responses to stress, even much later in life (i.e., three years after the abuse occurred). Clearly, early life experiences, particularly those of stress, can be advantageous in moderation (i.e., via "buffering" or stress-inoculation) but detrimental in great amounts (e.g., due to dysregulation of crucial systems like the HPA axis) and or very small amounts (i.e., never developing coping mechanisms to stress). When considered in combination with life history theory, experiences of stress are likely to inform an individual about future environmental conditions and have a hand on influencing the development of sexual maturity.

However, simply maturing into a sexually active adult is not sufficient for completing adolescence. There are a suite of behavioral, psychological, and physiological changes one must undergo, which show wide interindividual variation, yet the influences on this variation are still poorly understood. In the studies I describe in this thesis, I explore the influences of interindividual variation on sexual development and maturity in free-ranging rhesus macaques living on Cayo Santiago, Puerto Rico. While there has been some limited work done in captive settings with non-human primates investigating influences on sexual maturation, to date very little work has been conducted on individuals living under naturalistic conditions. Further, few studies have combined work to look at behavioral, psychological, and physiological development simultaneously. Together, the studies presented in this thesis combine to lay the groundwork for a comprehensive understanding of how social factors like dominance rank and experiences of early life stress affect life history trajectories of young females entering adolescence.

Chapter 2 investigates the both the environmental and social factors that affect age at first reproduction in young females. Although previous work in captive primates has revealed that dominance rank is an important influence on AFR (e.g., Wilson et al. 1984), dominance rank alone cannot explain the variation seen in AFR. One of the only studies on Cayo Santiago to look at variation in AFR comes from a demographic investigation by Blomquist (2012). Blomquist was able to model several characteristics across the lives of several generations of macaques living on Cayo Santiago, such as seasonal effects, birth order, dominance rank, and heritability, but he was not in the field conducting behavioral observations and can only speak to the influence of demographics on AFR. Interestingly, his models only explain about 33% of variation in AFR, demonstrating that nearly two-thirds of variation in AFR could be environmentally influenced.

I studied a cohort of female rhesus macaques from the same 2011 birth season, from birth through their adolescent mating season. My aims were to see how factors of physiological stress and early life adversity, measured as the amount of neglect and abuse mothers directed toward my subjects as infants, interacted with factors like dominance rank, which are known to influence AFR. I found that early life adversity had no effect on the sexual functioning of adolescent females as measured by sex hormone concentrations, but that, as expected, dominance rank was highly influential on AFR. Further, I found that latency to being cycling in the mating season was an important factor influencing AFR. Unsurprisingly, high ranking females were much more likely to get pregnant that females of other rank. Further, high ranking females had a much shorter latency to begin cycling. However, females of other lower ranks who did manage to have a short latency to begin cycling also were able to become pregnant. These results suggest that dominance rank's influence on AFR is unlikely to be directly influencing AFR, but rather moderating a female's body condition, which allows earlier menarche and pregnancy success. Presumably, some low ranking females were also able to achieve a body condition similar to those of high ranking females and were also able to achieve an earlier AFR.

While sexual behavior is the most obvious change that is to be expected upon reaching adolescence, there are a variety of social behaviors that must also change as one becomes an adult. Individuals who are becoming adults must quickly shift their behavior to match the completely new set of demands of adulthood, or risk failing to succeed socially (Spear 2000). Specifically, individuals are shifting from a mostly same aged peer group, to a widely varied adult social network. These changes can be extremely physically and psychologically stressful. Chapter 3, therefore, examined the changes in stress and social behavior from the juvenile to adolescent period. To study these changes, I investigated social behaviors and stress physiology and behavior in both the juvenile and adolescent mating season of the same cohort of female macaques as Chapter 2. My aims were to: 1) assess the change in behavioral and physiological stress indicators measured by self-directed behaviors, restlessness, and cortisol, 2) assess how social factors affect changes in stress during adolescence, 3) assess if changing social demands in adolescence alter social behaviors, and 4) investigate the role of progesterone on female social behavior.

Consistent with predictions, physiological stress levels increased from the juvenile to adolescent period, but behavioral indicators of stress were either unaffected or decreased. Further, dominance rank did affect restlessness behavior in the predicted direction and consistent with results seen in adult males. I also found evidence that adolescent individuals do respond to changing social demands of adulthood by increasing their grooming behavior, but only with other adult females. Finally, I found evidence that progesterone levels seem to be associated with female affiliative behavior. These data suggest that rhesus macaques experience physiological stress responses to the social changes associated with this developmental period. Further, they respond by altering social behavior to meet the demands of the new social network in specific ways. Long term social bonds have been shown to be beneficial in a variety of primate species (e.g., chacma baboons, Silk et al. 2010b; Assamese macaques, Haunhorst et al. 2016; Barbary macaques, Young et al. 2014; chimpanzees, Koski et al. 2012), including rhesus macaques (e.g., Brent et al. 2011). Because grooming behavior specifically increases only with adult females, this is not a general increase in the behavior produced with age, but a specific choice made by the adolescent individuals, possibly to gain the potential health benefits or to relieve the increased levels of stress.

While Chapter 3 focused on the changes in social behavior, which are necessary for joining the adult social network, there are a variety of other changes adolescent individuals must make in order to successfully engage in mating. The final two chapters of the thesis focus on both the production and perception of sexual signals. Sexual signaling of fertility is common across the animal kingdom, and come in a variety of modalities. In many primates, the dominant modality of signals is visual. Common signals that females will use to advertise their fertility status to males include skin color on the face or hindquarters (Dubuc et al. 2009) or swelling of the skin in the anogenital region (chimpanzees, Machatschke et al. 2006; baboons, Rigaill et al. 2013; crested macaque, Higham et al. 2012). In rhesus macaques, facial redness has been shown to be markedly higher in the fertile period than in the days prior to or following the fertile period

(Dubuc et al. 2009). Further, Higham et al. (2011) demonstrated that this is a meaningful signal to males. In their study, the authors used a looking time method of simultaneous photograph presentation of the same female taken either during or outside of her fertile period. The results indicate that males looked longer at the picture of a face taken during a female’s fertile phase than a picture taken outside that fertile period, demonstrating that the males could distinguish important information from the signals of the females. Even more importantly, males were only able to distinguish between pictures of a female’s fertile or not-fertile phase if the male was familiar with the female (i.e., lived with her in a social group for an extended period of time). This demonstrates the fact that there is a wide inter-individual variation when it comes to facial redness beyond the intra-individual variation of a female across her own cycle. However, adolescents in this species also display anogenital swellings. Anogenital swellings are common among females in their first mating season, but almost entirely absent in all other females. In many primates, adolescents will display an exaggerated version of sexual signals, and continue to display those signals in an attenuated manner throughout adulthood (reviewed in Anderson and Bielert 1994). However, the function of these exaggerated adolescent signals remains unclear. Anderson and Bielert (1994) propose a variety of possible functions including super-normal sexual stimulus to entice reluctant adult males, a prevention guarding against cross-species hybridization, a mechanism for female-female competition to limited male partners, to help integration into a new group (in female dispersing species), or it may simply be a nonadaptive side-effect of some other phenomenon (e.g., hyper reactivity to hormone stimulation for the first time). Most importantly, not all the explanations are mutually exclusive and can vary from one species to another, making it critical to examine the function of the signal in each species in the context of its socio-ecology. Beyond knowing that these hindquarter swellings represent a unique signal to adolescent individuals in rhesus macaques, the function of this signal remains unclear. As in many species, nulliparous rhesus females are less desirable mating partners because they are less likely to have a surviving offspring than multiparous individuals (Anderson 1986). The swelling signal in these females may serve a variety of functions, including attracting males who would otherwise overlook them for multiparous partners, signal actual time of ovulation as in other primate species (i.e., time peak swelling to ovulation), or be simply an overreaction to the first exposure of circulating estrogen. The goal of Chapter 4 was to elucidate the function of the adolescent swelling in rhesus macaques. I predicted that the swellings would align to the predictions of either the graded signal hypothesis (Nunn 1999) or the female quality indicator hypothesis (Domb & Pagel 2001). The graded signal hypothesis states that swellings give a probabilistic cue of the timing of ovulation, where speak swelling size is correlated with, but not a perfect indicator of, maximum fertility. Alternatively, the female quality indicator hypothesis states that swellings are energetically costly and therefore indicate high quality of a female's ability to conceive and carry a pregnancy to term. However, my results did not support either hypothesis: swellings did not peak in size during estrus, and males did become more interested in females with increasing swelling size. Given these results, the framework explaining sexual swellings in primates must be expanded to include the fact that adolescent rhesus macaques do not fit the framework explaining this signal in this taxa. However, given its presence only in adolescence, the first resurgence of the HPG axis, it is entirely possible that females are simply differentially reacting to newly circulating levels of female sex hormones.

Adolescent females are not only producing their own sexual signals for the first time in puberty, they are also becoming aware of signals sent from other members of the social group, namely the signals produced by males. Dubuc et al. (2016) investigated preferences of male facial color. Results indicate that both adult males and females prefer to look at faces that are redder, yet 16 month old juveniles do not distinguish between dark and pale faces. Therefore, while this signal is important to adults, it seems to be a bias that develops at some point in late juvenility or early adulthood. While the function of the facial redness ornament in males is not entirely known (e.g., it is not related directly to rank), these results in conjunction with previous working showing that redder males receive more sexual solicitations from females (Dubuc et al. 2014) indicate that male facial redness is an important cue to sexually active adults, both males and females, and not simply a general sensory bias. Therefore an important and unanswered question is at what point does this preference develop? It may simply be the result of a developing perceptual system (e.g., development of higher level visual cortices), response to new circulating hormones (e.g., spike in estrogen, progesterone, or testosterone in adolescent individuals), or a learned response to interacting with these adult males (e.g., after gaining experience mating with or fighting against males of different color). To investigate when a bias for looking at darker faces develops, I tested 130 2, 3, and 4 year old males and females during the mating season using the same methods as Dubuc et al. (2016). Interestingly, no age-sex category had a preference and looked equally at both darker and paler male faces, despite the fact that according the results of Dubuc et al. (2016), a preference, at least in some of the subjects (i.e., females) should have developed over some of these age groups. Further work will be needed to uncover when exactly in development preference for this signal emerges.

Together, these studies provide a comprehensive investigation of the sources of variation in sexual and social development of adolescent female rhesus macaques. The majority of the data presented here come from a single birth cohort of individuals, and so further work with more individuals in different years can confirm the conclusions I make here. However, given that the majority of results align with predictions from the human and captive primate literature, these studies illustrate that there is indeed wide variation in adolescent maturation, and that many factors are influential on behavioral and physiological development (Chapters 2 and 3), and that the variation in the development signal production (Chapter 4) and perception (Chapter 5) is still poorly understood and more work is needed to complete our understanding of these phenomenon in this nonhuman primate model.

CHAPTER 2

Predictors of age at first reproduction in adolescent female rhesus macaques

ABSTRACT

Age of first reproduction is the one of the most important factors that influences lifetime reproductive success. In female primates, there are a variety of factors that can affect the timing of menarche and subsequently age of first reproduction. Previous work has demonstrated that about 33% of variation in age of first reproduction is explained by parental rank, birth order, seasonal effects and heritability. This leaves a large portion of the variation in age of first reproduction explained by unknown environmental factors. This study investigated the effects of early life adversity, and its relationship with cortisol levels, on age at first reproduction. Results indicate that dominance rank and latency to cycle in the season were the most important factors affecting age of first reproduction, while early life adversity had no significant effect. Greater access to nutritional resources by females of high rank may be what moderates the rank related differences in age of first reproduction.

INTRODUCTION

Life history theory (LHT) is an evolutionary framework that attempts to explain how variation in multiple genetic and/or phenotypic characteristics are adaptive responses to maximize reproductive success (Stearns 1976). In general, animals have a limited amount of energetic resources to spend on three major areas: somatic growth, reproduction, and somatic maintenance/repair. Life history theory predicts that animals should vary how much they allocate to these various areas depending on the ecological constraints of a given individual at a given time. Further, LHT can be used to compare variation either between different species or within members of a single species. Among primates, LHT has been an important framework for helping explain interindividual variation in development. For example, one of the most important life history traits of female primates is age of first reproduction (AFR). AFR is one the greatest predictors of lifetime reproductive success in females (Walters 1987), yet we know little about what influences variation in AFR.

Much of the work investigating variation in AFR among female primates comes from studies of captive animals. Early work with laboratory housed female rhesus macaques (*Macaca mulatta*) housed in constant, controlled settings found no seasonal effects on the timing of menarche or rate of reproductive maturation (Resko et al. 1982). However, when Wilson et al. (1984) compared subjects living outdoors to those who had been laboratory housed before being transferred to outdoor facilities, they found evidence for seasonal effects on reproductive maturation in the females housed outdoors .

One area of research on variation in pubertal timing has focused on growth rates among infants and juveniles. For example, clinical work in humans has demonstrated that birth weight is an important factor influencing the timing of menarche, where girls with heavier birth weights tend to have early puberty compared to normal birth weight individuals, despite growth at similar rates (dos Santos et al. 2002). In a study on captive rhesus macaques, researchers found that the critical factor affecting reproductive maturation was not the birth weight of the animal, but its growth trajectory (Coe and Shirtcliff 2003). There is a major difference, however, between human and nonhuman primate growth rates, as underweight humans tend to go through a “catch-up” growth phase, whereas monkey growth rates are stable and sustained throughout development (Coe and Shirtcliff 2003). Therefore, investigating the factors affecting growth rates is also needed to fully understand differences in AFR.

A common social factor affecting many aspects of child development is socioeconomic status (SES). Many studies have demonstrated that low SES is associated with an earlier onset of puberty (Deardorff et al. 2014, James-Todd et al. 2010). SES is likely to affect growth rates, probably through access to resources, but the relationship is not straightforward because it depends on sociocultural context. For example, in a longitudinal study on Polish children born between 1966 and 2012, researchers studied the effects of SES on variation in height and BMI. They found that generally improving economic conditions of the country over time were associated with increases in both height and BMI. Further, high SES individuals tended to score higher on BMI than low SES individuals (Nowak-Szczepanska et al. 2016). However, in more industrialized countries, lower SES individuals tend to rely on inexpensive, high-caloric food that increases fat stores and obesity. For example, in a study of Canadian children, there was a significant relationship between low SES and obesity prevalence (Oliver and Hayes, 2005).

Similar to humans, nonhuman primates have strict social hierarchies of dominance that mirror SES in humans. Primates of higher rank have preferential access to resources, similar to high SES humans. Unsurprisingly, studies of captive primates have shown rank related differences in age of onset of puberty. For example, Zehr et al. (2005) demonstrate that in captive rhesus macaques, females from low ranked families had significantly delayed onset of ovulation compared to middle and high ranking individuals. Similarly, Wilson et al. (1983) demonstrated rank related differences in AFR for a captive colony of rhesus macaques. Only around 20% of their subjects experienced an early AFR, with the majority of those early developing individuals (75%) coming from high ranking families. In a study of free-ranging rhesus macaques, Blomquist (2012) investigated variation in AFR among the rhesus macaques of Cayo Santiago, using longitudinal demographic data. He demonstrated that about 33% of the variation in AFR among the female macaques was explained by parental rank, birth order, and heritability. However, this still leaves 67% of the variation explained by other unknown environmental factors.

While it is clear that growth trajectories and dominance rank are important factors affecting both the onset of puberty as well as AFR, there is still a large portion of variation of these factors that remains unexplained. One avenue that has promise to help explain this variation is maternal attachment and experiences of stress. For example, in girls, having a mother with a mood disorder predicted earlier puberty. However, this relationship was mediated by dyadic stress and, in cases where girls were not living with their biological father, by stepfather presence (Ellis and Garber 2000). The authors suggest that stepfather presence and stressful family experiences create two separate paths that lead to early pubertal debut in girls.

Most nonhuman primates are raised exclusively by their mother, and this social relationship is the most influential on subsequent development of the offspring, who relies on maternal input for information about ecological conditions. Maternal styles can be broken down across two different continuums: 1) rejecting/accepting, and 2) controlling/laissez-faire (Fairbanks 1996). Across these continuums, mothers often act in consistent, predictable ways towards their infants, such as restraining the infant to prevent it from leaving maternal proximity. These maternal behaviors often exert strong behavioral and physiological responses in infants, such as increased number of tantrums and increased basal cortisol levels (Maestripieri 2002, Mandalaywala et al. 2014). Indeed, early life adversity (ELA) related to the type of maternal care provided has been shown to have effects on neuroendocrine development (e.g., Sanchez et al.

2010; Petrullo et al. 2016). Research in this area focuses particularly on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis, which is the system that coordinates the stress response.

To date, there has been no research done to investigate how ELA and stress interact with social factors to affect AFR in free-ranging primates with what little work that has been done in the area having been conducted exclusively in captivity (e.g., Maestripieri 2005). Therefore the goal of this study was to study the interacting effects of maternal maltreatment and stress physiology on social factors to determine the environmental sources of variation in AFR of adolescent female rhesus macaques. I predicted that infants exposed to high ELA would have higher basal cortisol levels across the mating season than those exposed to low ELA. Further, I predicted that females exposed to high ELA would have delayed AFR compared to those exposed to low ELA. I also predicted that dominance rank would influence AFR such that high ranking females would have earlier AFR compared to those of low rank.

METHODS

Study Site and Subjects

This study was conducted on Cayo Santiago, a 15.2ha island located 1 km off the southeast coast of Puerto Rico. A rhesus macaque colony was established in 1938 with wild individuals captured in India (Rawlins and Kessler 1986). All animals are habituated to human presence. At the time of the study, the population on Cayo Santiago was approximately 1400 individuals separated into eight naturally formed social groups. The subjects of this study were 22 females born between August-September of 2011 from two of the eight social groups. The subjects were approximately equally distributed across dominance ranks (high: N = 5, middle: N = 8, low: N = 9).

Behavioral Observations

Behavioral data were collected five days a week from 0700-1430 hours via focal animal sampling (Altmann 1974). Each animal was observed for 30 minutes once a week. Focal follows were counterbalanced bi-weekly between morning (0700-1030 hours) and afternoon (1030 to 1430 hours) sessions to control for diurnal effects on behavior. The types of behavior collected include affiliative (e.g., grooming), agonistic (e.g., threats, submission), sexual (e.g., copulation, solicitation), and self-directed (e.g., scratching, yawning). Appendix 1 contains the full ethogram with all behavioral descriptions.

For interactive behaviors (e.g. grooming) individual ID of the partner was recorded if possible, otherwise the age-sex class of the interactant was used (e.g. juvenile male, adult female). Further, the directionality of all behaviors was recorded (e.g. whether the subject was grooming or being groomed). For non-interactive behaviors, the nearest neighbor within 1m is recorded. All data were recorded using the Behaviour software on a Psion Workabout. Data were parsed into an Access database (Microsoft Corp., Redmond, WA, USA) and queries were used to obtain frequency and duration of behaviors.

In addition to focal animal follows, all subjects were surveyed four times daily to assess estrus status. Females were considered to be in behavioral estrus if they were seen mating, soliciting males, or if they had a sperm plug.

Assessment of Early Life Adversity

All subjects were part of a larger dataset for a previous project in the Behavioral Biology Laboratory which collected behavioral data on mother-infant interactions for the first 12 weeks of life. In these first 12 weeks, the subjects were video recorded for 30 minutes twice a week. Videos were coded for mean hourly rates of maternal rejection and abuse (see ethogram). Abuse and rejection rates were averaged across the 12-week study period to obtain a single value for overall early life adversity (ELA), and subjects were binned into 3 categories: high, moderate, and low. These categories have been shown to be behaviorally and physiologically valid (see Mandalaywala et al. 2014).

Fecal sample collection and hormone assessment

All subjects had opportunistic fecal samples collected from them every 2-3 days in order to assess concentrations of estrogen, progesterone, and cortisol. Previous work in this population has showed that fecal samples collected from the same individual in the morning and afternoon show no differences in hormone concentrations (Rahkovskaya & Heistermann, n.d.). A total of 462 fecal samples were collected from all 22 subjects. On average each female had 21.0 ± 0.81 samples collected across the 15 week study period, or 1.4 ± 0.05 samples per individual per week.

Fecal samples were collected only after defecation was observed directly and the sample was uncontaminated with urine. Samples were subsequently homogenized, removing any nonfecal debris, and a small bolus of approximately 1.0g was placed in a 20ml tube. These tubes were stored in insulated coolers containing ice packs. This method has been validated in the field as reliably storing samples until they can be brought into a freezer without altering hormone concentrations (Hodges & Heistermann, 2011). Upon returning from the field, fecal samples were frozen at -20^{o}C until they were shipped on dry ice to the Primate Reproductive Ecology and Evolution lab at NYU. All samples arrived frozen. Fecal samples were prepared for Enzyme-

Immuno Assay (EIA) by being lyophilized and pulverized followed by extraction of an aliquot (50-70 mg) of the fecal powder with 3 ml 80% methanol by vortexing for 15 min (Higham et al., 2009). After fecal extractions were performed, the samples were shipped to the German Primate Center at the University of Göttingen, where the EIAs were carried out.

Progesterone and estrogen concentrations were measured by pregnanediol-3-glucuronide (iPdG) and total estrogens (iE_{total}) metabolites respectively, both of which have been validated for use in macaques (Engelhardt et al. 2004). Intra- and interassay coefficients of variation, determined by replicates of high and low value quality controls. Progesterone metabolite interassay variation had values between 4.6% (high) and 6.9% (low), and intra-assay values between 4.1% (high) and 6.2% (low). Estrogen metabolite interassay variation had values between 10.9% (high) and 8.8% (low), and intra-assay values between 3.0% (high) and 6.3% (low).

Cortisol levels were assessed using 5β-reduced 3α,11β-dihydroxylated cortisol metabolites, which has been shown to be a major cortisol metabolite in several primate species (Heistermann et al. 2006), including rhesus macaques (e.g., Mandalaywala et al. 2014, Hoffman et al. 2011). Again, intra- and interassay coefficients of variation, determined by replicates of high and low value quality controls. Interassay variation had values between 10.2% (high) and 14.9 % (low), while intra-assay variation had 3.8% (high) and 5.2% (low).

All assays are standardized for differences in fecal weight and are expressed as concentrations (ng) per dry fecal weigh (g). To achieve normal distribution, hormone concentrations were log transformed for all analyses.

Data Analyses

In order to assess the effect of early life adversity on hormone levels, Linear Mixed Models (LMMs) were used; each hormone was analyzed separately as the dependent variable, the ELA category as fixed factor, and the individual animal ID as a random effect to control for repeated observations. Similarly, in order to assess the effect of dominance rank on hormone levels, each hormone was analyzed separately as the dependent variable, dominance rank as fixed factor, and the individual animal ID as a random effect to control for repeated observations. Cortisol values were log transformed before analysis to achieve a normal distribution.

In order to assess if ELA had an effect on the eventual pregnancy status of a female, a nonparametric Kruskal-Wallis test was run using ELA categories as the grouping variable, and the eventual pregnancy status of the female as the dependent variable. In order to assess if dominance rank affected AFR, a Chi-Square goodness of fit test was used to see if females of different rank became pregnant more or less than expected by chance.

Finally, I tested whether ELA or dominance rank was influential on the latency to begin cycling in the mating season. For both tests, a nonparametric Kruskal-Wallis test was run using ELA and dominance rank as the grouping variables, and the latency to begin cycling as the dependent variable. Finally, a nonparametric Mann-Whitney test was used to test for a difference between latency to cycle between females who became pregnant and those who did not.

All tests were run in IBM SPSS v. 23.0. All tests were run as two-tailed tests with the alpha level set as $p \leq 0.05$.

RESULTS

Early Life Adversity

Experiences of early life adversity had no significant effect on cortisol levels in adolescent females prior to the mating season (LMM: $F_{2,19.163} = 0.731$, $p = 0.494$). Similarly, there was no significant effect of experiences of early life adversity on cortisol levels across the mating season (LMM $F_{2,18.988} = 0.075$, $p = 0.928$). Early life adversity, further, did not influence the levels of circulating sex hormones (LMM progesterone: $F_{2,13.496} = 1.441$, $p = 0.271$; estrogen: $F_{2,14.959} = 0.566$, $p = 0.579$), even when controlling for estrus status. Experiences of early life adversity did not influence eventual pregnancy status (Kruskal-Wallis $\chi^2 = 0.017$, df = 2, $p = 0.991$). Further, experiences of early life adversity did not influence latency to begin cycling in mating season ($\chi^2 = 0.640$, df = 2, $p = 0.726$).

Dominance Rank

There was a significant effect of rank on estrogen levels (LMM $F_{2,19.112} = 4.127$, $p = 0.032$). On average, high ranking females had higher estrogen levels than middle and low ranking females across the mating season. However, when controlling for pregnancy this effect is no long significant (LMM $F_{2,23.684} = 1.643$, $p = 0.215$).

There was a significant effect of rank on age of first reproduction ($\chi^2 = 10.05$, df = 2, $p < 0.01$). High ranking females were much more likely to become pregnant than middle or low ranking females (see Figure 2.1). Further, there was a significant effect of rank on the latency to begin cycling (Kruskal-Wallis $\chi^2 = 7.682$, df = 2, $p = 0.021$). High ranking females began to cycle much earlier in the mating season (mean = 4.80 ± 1.11 weeks) than middle (mean = 9.17 ± 0.60 weeks) or low ranked (mean = 9.13 ± 0.55 weeks) females (Figure 2.2).

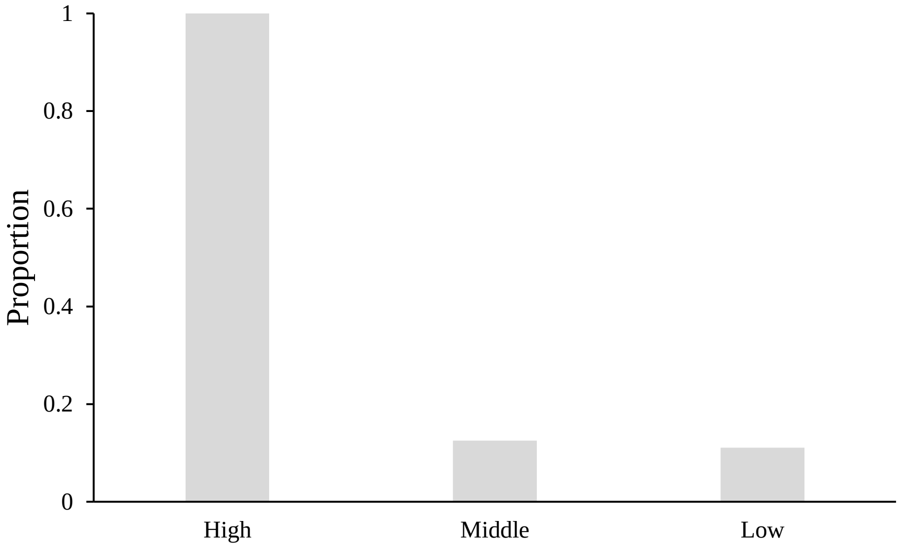

Figure 2.1. The proportion of high: 1.0, middle: 0.125, and low: 0.111 ranked females that became pregnant.

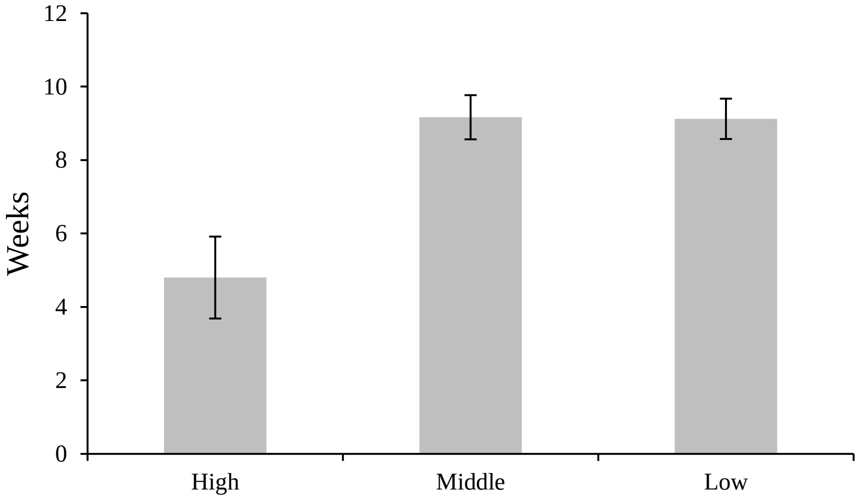

Figure 2.2. The average latency in weeks (± SEM) for high: 4.8 ± 1.11, middle: 9.17 ± 0.60, and low: 9.13 ± 0.55 ranked females to first cycle in the mating season.

Latency to Cycle

There was a significant effect of latency to cycle on the eventual pregnancy status of a female (Mann-Whitney U = 3.500, N_1 = 12 N_2 = 7, p = 0.001). Females who eventually became pregnant started cycling earlier in the season (mean = 5.29 ± 0.84 weeks) than those who did not become pregnant (mean = 9.58 ± 0.29 weeks; Figure 2.3).

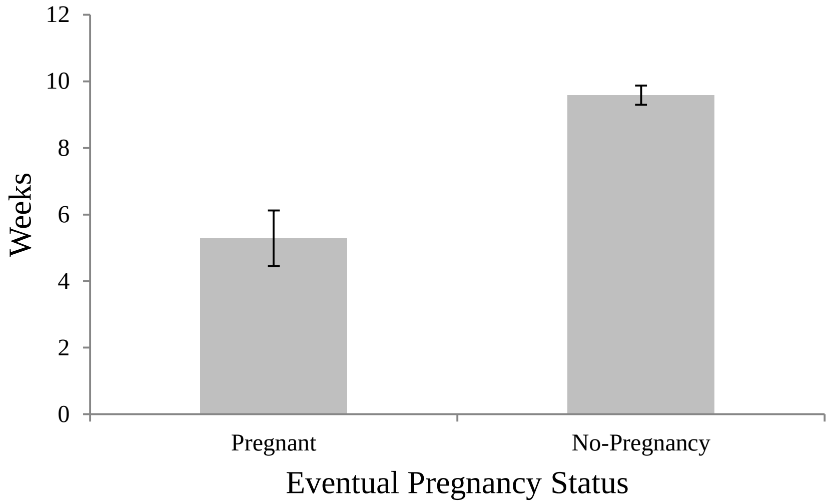

Figure 2.3. The average latency in weeks (± SEM) for a female to begin to cycle for females who eventually become pregnant: 5.29 ± 0.84 and those that did not: 9.58 ± 0.29.

DISCUSSION

Based on the data presented here in this study, experiences of early life adversity do not influence the age of first reproduction in rhesus macaques. This is in direct contrast to evidence from humans which suggests that being raised in a stressful environment is predictive of having earlier puberty (e.g., Ellis and Garber 2000). Indeed, not only was there no effect of early life adversity on age of first reproduction, but ELA also had no noticeable differences on the functioning of the HPG axis. The female sex hormones were not different between females who experienced various amounts of ELA, again different than what has been suggested in the human literature (Trickett & Putnam 1993). Other evidence from this population has suggested that ELA does influence the regulation of the HPA axis (Petrullo et al. 2016), such that individuals who experienced high ELA had blunted salivary cortisol responses to acute stressors, yet higher baseline cortisol levels in nonstressful periods, suggesting that individuals who were maltreated as infants have dysregulated stress response systems. One major difference between previous research and the data presented here is that Petrullo et al. (2016) were able to assess HPA function with more temporally precise measures of salivary cortisol and salivary alpha-amylase in response to a specific behavioral trigger, an agonistic interaction. In this study, hormone concentrations were measured via fecal samples, representing a more global measure of hormone levels across a larger period of time. As such, it is possible to assess estrus state, but not to precisely time the ovarian cycle. Perhaps with more fine-tuned measurements documenting the cycle more precisely, one might be able to see if experiences of early life adversity are affecting the functioning of the HPG axis.

The data presented here replicate the finding that dominance rank is an important factor affecting age at first reproduction. Of the 22 subjects in the study, all of the high ranking females became pregnant, while only one middle and one low ranking female became pregnant. Further, on average the high ranking females started sexually cycling significantly earlier in the season than did middle or low ranking females. Perhaps one of the most interesting results was that on average, all females who eventually became pregnant started cycling earlier in the season than did females who cycled but did not become pregnant. These results seem to indicate that there is some reproductive advantage for cycling earlier in the season. However, this is not necessarily the case for all females, as older, non-adolescent females of this population are known to get pregnant at all times across the 6 month mating season (Cayo Santiago Census Data). Therefore, among adolescent females there must be some individual characteristics which influence both the ability to begin cycling earlier in the season and the ability to conceive successfully. While all the high ranking females fulfilled these two conditions, it was not exclusive to females of high rank as it also occurred, though rarely, in some middle and low ranking females. One possible explanation is a difference in energetic status. On Cayo Santiago there is a period of lowered food availability for colony management purposes in the two months immediately prior to the mating season. During this time, rank related differences in ability to acquire resources are most apparent, and middle and low ranking females are much less likely to feed as much as they normally would. In humans, energetic status is known to have an effect on the onset of menarche (Leenstra et al. 2005) as well as on maintaining normal ovarian cycles (Ellison 2003). If high ranking females are using their social advantages to monopolize the limited food resources immediately prior to the mating season, this could explain why all of the high ranking females had the necessary energetic stores to achieve an early onset of menarche as well as gestate to full term. Since high ranking females are unable to completely monopolize the food resources of the island, this could explain why some (though significantly fewer) females of middle and low rank were also able to achieve a seasonally early menarche and full term pregnancy. Further research in this area should focus on collecting data related to energetic status of females prior to and throughout their first mating season. One candidate for study would be the recently validated urinary c-peptide (e.g., Girard-Buttoz et al. 2011), which has been shown to be a reliable biomarker of body and nutritional status. Another possible avenue of research would be with the hormone leptin. Serum levels of leptin have been shown to be a reliable marker of body mass and fat stores in apes (Bribiescas & Anestis 2010) and humans (Bribiescas 2001). However, leptin has yet to be validated in urine for catarrhine primates. Therefore, while the measure could be an extremely helpful tool for further research of variation in AFR in free-ranging macaques, the measure needs to first be validated for use in this species.

Overall, this study confirms that in free ranging populations of rhesus monkeys, dominance rank is an important factor determining age at first reproduction in adolescent females. While the exact mechanism underlying this effect remains unclear, it is likely that dominance rank is affecting access to nutritional resources, such that high ranking females had a better body condition at the onset of the mating season, allowing them to start cycling earlier. Further, the fact that all females who became pregnant, regardless of their rank, began cycling significantly earlier thank those females who were hormonally cycling but never successfully conceived, seems to indicate that the benefits of high social rank do not exclusively explain the differences in AFR and potentially indicate that body condition is an important factor. Future studies should focus on assessing body condition prior to and throughout the mating season across all dominance ranks to explain the exact mechanism determining age of first reproduction in this and other species.

CHAPTER 3

Changes in social behavior during the transition from juvenility to adolescence in female rhesus macaques

ABSTRACT

Adolescence is the one of the most critical developmental periods in mammalian development, accompanied by major psychological and physiological changes. Individuals must quickly adapt to new behavioral demands in order to succeed as adults in the social network, and these demands may be highly stressful. In female primates, the adolescent period presents an opportunity to begin forming social bonds with adult group mates which may convey life-long health benefits and help attenuate the new stressors they face. This study investigated the behavioral changes in stress physiology and behavior, as well as affiliative social behaviors in a cohort of rhesus macaque females across their juvenile and adolescent mating seasons. Results indicate that, similar to humans, rhesus macaques show increased cortisol levels in adolescence, though have a decrease or no change in behavioral indicators of stress. Results also show that, as predicted, rates of affiliative social behaviors, measured by grooming, increase during the adolescent period. Further, progesterone concentrations seem to influence the rate at which females engage in grooming behavior. However, these increases in grooming behavior are specifically only with other adult females, and show no changes in grooming behavior with adult males or juveniles. These results may indicate that adolescent female rhesus macaques attenuate the stress of the adolescent period by forming social bonds with other adult females, possibly in line with Taylor's (2000) tend-and-befriend theory of stress response. Further, results may

indicate that progesterone in macaques, like humans, may facilitate a motivation for affiliative behavior.

INTRODUCTION

Adolescence is a transitional development period during which individuals undergo many physical, physiological, and psychological changes. This transition period is critical for development because it allows individuals time to gain the skills that are necessary to successfully integrate as fully adult members of their social groups. However, this period can also be extremely stressful, both psychologically and physically, due to all the rapid social and physiological changes that the body undergoes during this time. Individuals must quickly shift their behavior to match the completely new set of demands of adulthood (Spear 2000). For example, play behavior is uniform in juveniles across all primate species. Playing is an important part the life of developing primates, however, it declines significantly in adolescence and is absent in adulthood (Walters 1987). Therefore, while play behavior may play an important role in the physical and/or social development of juvenile individuals, the skills that it develops are no longer as important for the transition to adulthood and the behavior is largely abandoned. Instead, adolescent primates must learn to integrate themselves into the social, mating, and dominance networks of their groups.

It is to be expected that these rapid social changes would be accompanied by increased experiences of stress, and differences in stress responses (e.g., Del Giudice et al. 2011). While female primates in female philopatric societies begin learning about their relative rank from their mother at a young age (e.g. Berman 1980), it is only during adolescence that individuals can start reversing formerly submissive relationships towards adults as they attain their own adult body size (Holekamp & Smale 1991), and begin to form a definitive rank in the hierarchy. Therefore, navigating and defining completely new set of social relations is likely to have an impact on overall stress levels.

Alternative to the new stressor of forming one's dominance rank, one of the most important aspects of adult life in group living primates is the formation of social bonds. Many species of primates are known to exhibit strong, lasting social bonds over long periods of time (e.g., chacma baboons, Silk et al. 2010b; Assamese macaques, Haunhorst et al. 2016; Barbary macaques, Young et al. 2014; chimpanzees, Koski et al. 2012). Social bonds have been shown to be beneficial to individuals in a variety of ways. For example, in Barbary macaques (*Macaca sylvanus*), the strength of male social bonds predicts the ability of an individual to recruit coalition partners for aggression, where stronger social bonds result in greater recruitment success (Young et al. 2014). Further, stable social bonds have been shown to have multiple health benefits. Silk et al. (2010a) demonstrated that female chacma baboons (*Papio hamadryas ursinus*) who have stronger and more stable social bonds with other females live significantly longer than females with fewer or weaker social bonds. Similarly, Brent et al. (2011) showed that in female rhesus macaques (*Macaca mulatta*), females with stronger, more focused social bonds had lower fecal glucocorticoid levels, implying that individuals with greater social capital are better at coping with stressors. Social bonds are clearly an important aspect of adult life with far-reaching consequences, and individuals in adolescence must therefore start forming these bonds in order to reap the lifelong benefits associated with such associations.

A relatively new area of interest in human behavioral endocrinology has focused on the role of progesterone in social bonding. For example, in one study subjects were primed with movie clips promoting affiliative motivation, power motivation, or a neutral clip. Results demonstrated that subjects' progesterone levels increased only after watching the affiliative motivating clip but not the others (Schultheiss et al. 2004). In another study testing the effects of social exclusion, Maner et al. (2010) found that subjects with high social anxiety display a significant decrease in progesterone levels following social exclusion, indicating a lack of affiliative motivation. In contrast, subjects with high rejection sensitivity had a significant increase in progesterone levels after given the opportunity to reaffiliate, which indicates a desire to engage in compensatory social affiliation. Further, Maner and Miller (2014) investigated the role of changing progesterone levels over the menstrual cycle of women and found that women in the luteal phase of their cycle (i.e., the phase of greatest progesterone levels) had increased accuracy decoding facial expressions and increased attention to social stimuli. Finally, in a study by Brown et al. (2009), the researchers manipulated the social interaction between dyads of women, with half the subjects performing a task promoting affiliation between the members of the dyad, while the other half participated in a non-affiliative task together. Individuals who participated in the affiliation task had higher levels of progesterone compared to those in the neutral task.

Despite the similarities in the endocrinology of humans and nonhuman primates, little work has been conducted to investigate the role of progesterone in prosocial behavior in nonhuman primates. A majority of the focus has been on the role of progesterone during pregnancy and immediately postpartum on infant directed behaviors, and little on social behaviors directed at others. However, Maestripieri (1999), investigated the role of circulating estrogen and progesterone on affiliative, aggressive, and sexual behavior in pig-tailed macaques (*Macaca nemenstrina*). Results indicate that as progesterone levels decreased across pregnancy, grooming interactions with other individuals also decreased in both frequency and duration. In a study of captive rhesus macaques, Maestripieri and Megna (2000) measured hormone levels and social behaviors across late pregnancy and early lactation. The results indicate a general reduction in grooming and increase in aggressive behavior across the transition from pregnancy to lactation. However, they also found that a greater reduction in the estrogen to progesterone ratio in lactation was associated with a smaller reduction in grooming behavior.

To date, little work has been done investigating the behavioral changes associated with the transition from juvenile to adolescent in non-captive primates. Given that adolescence represents major social changes, it is likely that social behaviors will be markedly different between individuals during their juvenile and adolescent years. Further, given the drastic change in hormone levels during the adolescent period, behaviors influenced by hormones are likely to undergo considerable changes as well. Therefore, the goal of this study was to investigate the changes in social behavior between the juvenile and adolescent periods of free-ranging rhesus macaques.

Consistent with the idea that adolescence marks a stressful period of rapid changes in social identity, there should be an increase of both physiological and behavioral stress indicators. Specifically, I predicted that cortisol concentrations, rates of self-directed behaviors, and restlessness would increase from the juvenile to adolescent period. Further, I predicted that social factors may moderate these stress behaviors in the adolescent period. Specifically, I predicted that high dominance rank and low early life adversity would lead to lower levels of restlessness and self-directed behaviors. Consistent with the idea that long-term social bonds can be formed in adolescence and generate life-long health benefits, I predicted that grooming rates would increase from the juvenile to adolescent period to facilitate the formation of long term social bonds. Finally, given the work in humans suggesting that progesterone is important for female affiliative behaviors, I predicted that higher progesterone levels would increase grooming rates of adolescent females.

METHODS

Study Site and Subjects

This study was conducted on Cayo Santiago, a 15.2ha island located 1 km off the southeast coast of Puerto Rico. A rhesus macaque colony was established in 1938 with wild individuals captured in India (Rawlins and Kessler 1986). All animals are habituated to human presence. At the time of the study, the population on Cayo Santiago was approximately 1400 individuals separated into eight naturally formed social groups. The subjects of this study were 22 females born between August-September of 2011 from two of the eight social groups. The subjects were approximately equally distributed across dominance ranks (high: N = 5, middle: N = 8, low: N = 9).

Behavioral Observations

The subjects were observed in both the 2014 and 2015 mating seasons. Due to changes in colony management practices, the 2014 observations occurred for 10 weeks from March 1 through May 7, while the 2015 observations occurred for 15 weeks from January 13 through April 26. Behavioral data were collected five days a week from 0700-1430 hours via focal animal sampling (Altmann 1974). Each animal was observed for 30 minutes once a week. Focal follows were counterbalanced bi-weekly between morning (0700-1030 hours) and afternoon (1030 to 1430 hours) sessions to control for diurnal effects on behavior. The types of behavior collected include affiliative (e.g., grooming), agonistic (e.g., threats, submission), sexual (e.g., copulation, solicitation), self-directed (e.g., scratching, yawning), and state (e.g. resting, traveling). Appendix 1 contains the full ethogram with all behavioral descriptions.

One behavior of interest here is restlessness. Restlessness is a behavior that is not observed directly, but rather a calculation of the rate of change of behavioral states. Therefore, restlessness is calculated as the hourly rate an individual changes between any of the following behaviors: rest, feed/forage, groom, self-groom, play, or travel.

For interactive behaviors (e.g. grooming) individual ID of the partner was recorded if possible, otherwise the age-sex class of the interactant was used (e.g. juvenile male, adult female). Further, the directionality of all behaviors was recorded (e.g. whether the subject was grooming or being groomed). All data were recorded using the Behaviour software on a Psion Workabout. Data were parsed into an Access database (Microsoft Corp., Redmond, WA, USA) and queries were used to obtain frequency and duration of behaviors.

In the 2014 mating season, all females were all pre-pubescent and none were ever seen to be sexually mature or active, with the average age of the subjects 2.50 ± 0.01 years at the beginning of the observation period. However, in the 2015 mating season all subjects could potentially have entered adolescence, with the average age of subjects 3.37 ± 0.01 years at the beginning of the observation period. Therefore, in addition to the focal animal follows in the 2015 season, all subjects were also surveyed four times daily to assess estrus status. Females were considered to be in behavioral estrus if they were seen mating, soliciting males, or if they had a sperm plug.

Assessment of Early Life Adversity

All subjects were part of a larger dataset for a previous project in the Behavioral Biology Laboratory which collected behavioral data on mother-infant interactions for the first 12 weeks of life. In these first 12 weeks, the subjects were video recorded for 30 minutes twice a week. Videos were coded for mean hourly rates of maternal rejection and abuse (see ethogram). Abuse and rejection rates were averaged across the 12-week study period to obtain a single value for overall early life adversity (ELA), and subjects were binned into 3 categories: high, moderate, and low. These categories have been shown to be behaviorally and physiologically valid (see Mandalaywala et al. 2014).

Fecal sample collection and hormone assessment

All subjects had opportunistic fecal samples collected from them every 2-3 days in order to assess concentrations of estrogen, progesterone, and cortisol. Previous work in this population has showed that fecal samples collected from the same individual in the morning and afternoon show no differences in hormone concentrations (Rahkovskaya & Heistermann, n.d.). A total of 462 fecal samples were collected from all 22 subjects in the 2015 season. On average each female had 21.0 ± 0.81 samples collected across the 15 week study period, or 1.4 ± 0.05 samples per individual per week. For the 2014 season, only a total of 39 samples were collected from 21 of the subjects. Each female had on average 1.72 ± 0.15 samples collected across the observation period.

Fecal samples were collected only after defecation was observed directly and the sample was uncontaminated with urine. Samples were subsequently homogenized, removing any nonfecal debris, and a small bolus of approximately 1.0g was placed in a 20ml tube. These tubes were stored in insulated coolers containing ice packs. This method has been validated in the field as reliably storing samples until they can be brought into a freezer without altering hormone concentrations (Hodges & Heistermann, 2011). Upon returning from the field, fecal samples were frozen at -20^{o}C until they were shipped on dry ice to the Primate Reproductive Ecology and Evolution lab at NYU. All samples arrived frozen. Fecal samples were prepared for Enzyme-Immuno Assay (EIA) by being lyophilized and pulverized followed by extraction of an aliquot (50-70 mg) of the fecal powder with 3 ml 80% methanol by vortexing for 15 min (Higham et al., 2009). After fecal extractions were performed, the samples were shipped to the German Primate Center at the University of Göttingen, where the EIAs were carried out.

Progesterone concentrations were measured by pregnanediol-3-glucuronide (iPdG) and total estrogens (iE_{total}) metabolites, which has been validated for use in macaques (Engelhardt et al. 2004). Intra- and interassay coefficients of variation, determined by replicates of high and low value quality controls. Progesteron metabolite interassay variation had values between 4.6% (high) and 6.9% (low), and intra-assay values between 4.1% (high) and 6.2% (low).

Cortisol (fGCM) levels were assessed using 5β-reduced 3α,11β-dihydroxylated cortisol metabolites, which has been shown to be a major cortisol metabolite in several primate species (Heistermann et al. 2006), including rhesus macaques (e.g., Mandalaywala et al. 2014, Hoffman et al. 2011). Again, intra- and interassay coefficients of variation, determined by replicates of high and low value quality controls. Interassay variation had values between 10.2% (high) and 14.9 % (low), while intra-assay variation had 3.8% (high) and 5.2% (low).

All assays are standardized for differences in fecal weight and are expressed as concentration (ng) per dry fecal weigh (g). Hormone values were positively skewed, and to achieve a normal distribution were log transformed for all analyses.

Data Analyses

In order to assess the change in cortisol concentrations, rates of self-directed behaviors, and restlessness from the juvenile to adolescent period, each female was assigned a single value of her seasonal average for each variable. Values were compared using the non-parametric Wilcoxon Signed Ranks Test. In order to assess how social characteristics influenced behavioral restlessness and self-directed behaviors in the adolescent year, Linear Mixed Models (LMMs) were used. For the LMM, restlessness and self-directed behavior rates were assessed as separate models as the dependent variable, while rank and ELA category were used as fixed factors. Individual animal ID was used as a random factor to control for repeated observations.

In order to assess the change in grooming behavior from the juvenile to adolescent period, each female was assigned a single value of her seasonal average time spent grooming with each age-sex category (i.e., adult males, adult females, and juveniles). Values were compared using the non-parametric Wilcoxon Signed Ranks Test. In order to assess how social characteristics influenced grooming behavior in the adolescent year, LMMs were again used. Grooming rates with each age-sex class were assessed as separate models as the dependent variable, while rank and ELA category were used as fixed factors. Individual animal ID was used as a random factor to control for repeated observations

In order to assess the influence of progesterone concentrations on grooming behavior during the adolescent period, LMM was used, with grooming rates with each age-sex class as separate dependent variables, while progesterone concentration was a fixed factor. Individual animal ID and estrus status was used as a random factor to control for repeated observations.

All tests were run in IBM SPSS v. 23.0. All tests were run as two-tailed tests with the alpha level set as $p \leq 0.05$.

RESULTS

Changes in stress related behaviors and physiology

The transition from juvenile to adolescent period significantly affected cortisol levels (Wilcoxon $Z = 2.520$, $p = 0.012$). Average cortisol concentrations were higher in the adolescent ($\bar{X} = 2.755 \pm 0.03$) period than in the juvenile ($\bar{X} = 2.631 + 0.04$) period (Figure 3.1). Further, the transition from juvenile to adolescent period significantly affected restlessness as well ($Z = 4.107$, $p < 0.001$). However, the pattern was in the opposite pattern as predicted. Average restlessness scores decreased from the juvenile ($\bar{X} = 101.30 \pm 3.11$) to adolescent ($\bar{X} = 73.46 \pm 3.27$) season (Figure 3.2). However, the transition between juvenile and adolescent period did not significantly affect the rates of self-directed behaviors ($Z = 0.308$, $p = 0.785$).

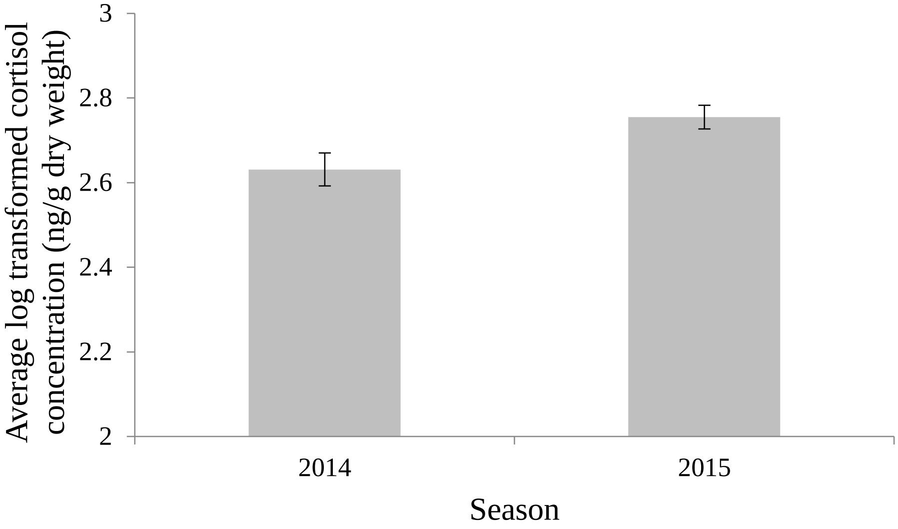

Figure 3.1. The average cortisol concentrations for females in the juvenile (2014) and adolescent (2015) season. Error bars represent ± SEM.

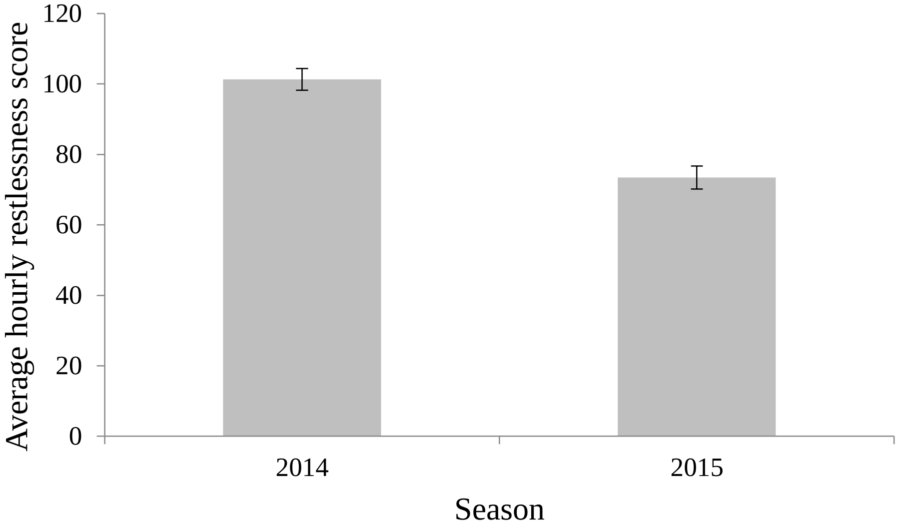

Figure 3.2. The average restlessness scores for females in the juvenile (2014) and adolescent (2015) season. Error bars represent ± SEM.

Within the adolescent season only, restlessness was affected by dominance rank (LMM F = 4.171, p = 0.033) but not by early life adversity (F = 0.313, p = 0.735). The pattern was as predicted, where high ranking individuals were less restless (X̅ = 58.82 ± 4.07) than middle ranking individuals (X̅ = 72.44 ± 3.82) who were in turn less restless than low ranking (X̅ = 82.29 ± 3.41) individuals (Figure 3.3). However, self-directed behaviors were not influenced by either rank (F = 0.474, p = 0.623) or ELA (F = 0.602, p = 0.549).

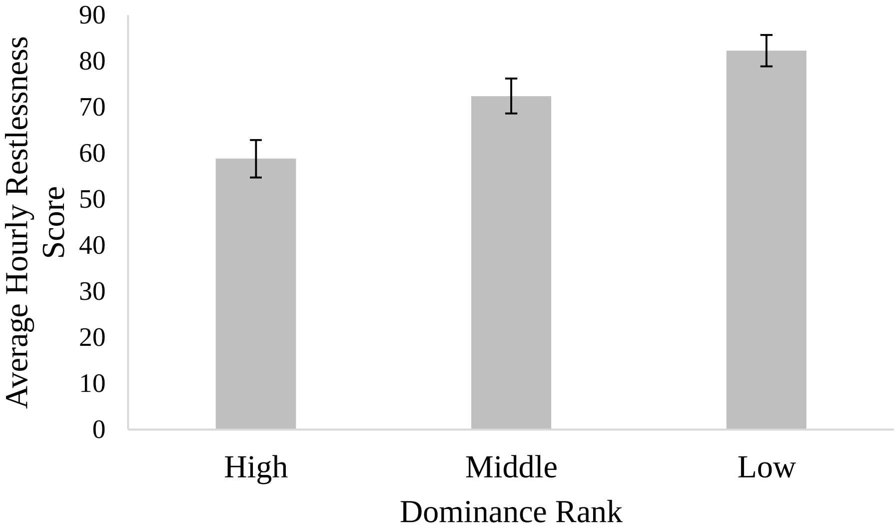

Figure 3.3. The average restlessness scores for females of different rank in the 2015 season. Error bars represent ± SEM.

Changes in grooming behavior

The transition from juvenile to adolescent period significantly affected grooming behavior. However, only rates of grooming with adult females were different across seasons (Wilcoxon Z = 3.652, p < 0.001), while grooming with adult males (Z = 0.166, p = 0.868) and juveniles (Z = 0.958, p = 0.338) remained the same. With adult females, as predicted the hourly proportion of time spent grooming significantly increased from the juvenile (X̅ = 1.57 ± 0.36) to the adolescent (X̅ = 8.51 ± 0.99) period (Figure 3.4).

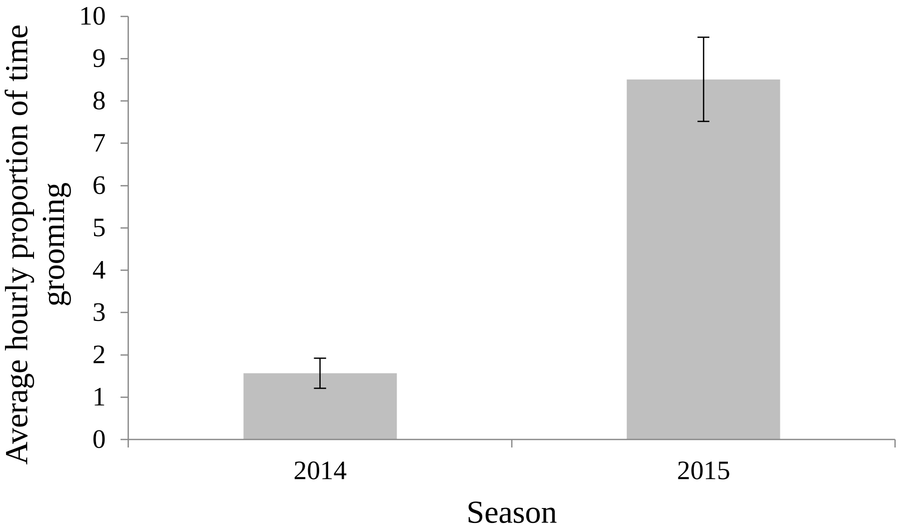

Figure 3.4. The average hourly proportion of time females spent grooming adult females in the juvenile (2014) and adolescent (2015) season. Error bars represent ± SEM.

Within the adolescent season only, grooming with adult females was not significantly affected by either dominance rank (LMM, F = 0.011, p = 0.989) or ELA (F = 0.216, p = 0.808). Further, grooming with adult males was also not affected by either dominance rank (LMM, F = 0.569, p = 0.577) or ELA (F = 0.406, p = 0.673).

Influence of progesterone on social affiliation

Within the adolescent season, grooming behavior was significantly influenced by progesterone concentrations. However, only grooming with adult females was significantly influenced by progesterone behavior (LMM, F = 1.783, p = 0.001), where increased progesterone levels was associate with increases in grooming behavior. Grooming behavior with adult males was not significantly influenced by progesterone concentrations (F = 0.557, p = 0.996), nor was grooming with juveniles (F = 0.683, p = 0.963).

DISCUSSION

Based on the data presented in this study, adolescent individuals alter their social behavior in specific ways to reflect new positions in the social network of their groups. Further, the data tentatively suggest that these experiences of change are stressful. My results mirror those seen in human children and adolescents (e.g., Kiess et al. 1995, Shirtcliff et al. 2012) where cortisol levels are significantly increased in adolescence. However, these results must be considered preliminary because of the limited number of samples collected per female in the juvenile period. However, due to their convergence with the human literature, this seems to suggest that rhesus macaques undergo a similar experience of physiological stress responses during their transition into adulthood, indicating that this species is a good model for studies of stress responses during adolescence. Future research should focus on collecting more cortisol samples in the juvenile period in order to replicate the results I present here.

In addition to the physiological stress responses, the adolescent period also significantly affected restless behavior. However, unlike cortisol which increased between the seasons, restlessness decreased during adolescence, which was opposite of the predicted pattern. Previous research in humans has linked restlessness and fidgeting to anxiety (e.g., Mehrabian & Friendman 1986). Further, research studying fidgeting in rhesus macaques has shown it is linked with boldness and risk-taking (Coyne et al. 2015). As adolescence is a period typically associated with greater risk-taking behavior (e.g., Reyna & Farley 2006), it was assumed that this behavior would also increase in adolescence. However, restless in the adolescent year only, did occur in the pattern predicted. Restlessness was highest in low ranking individuals and lowest in high ranking individuals (Figure 3.3). This pattern is consistent with that seen in restlessness of adult males (Higham et al. 2011). Therefore, stress reactivity in adolescent rhesus macaque females overall follows the predicted patterns.

Interestingly, unlike cortisol concentrations and restlessness, self-directed behaviors were unaffected by the transition from juvenile to adolescence, despite the fact that self-directed behaviors have been shown to reliably indicate experiences of psychosocial stress, increase in individuals given anxiogenic drugs, and decrease in individuals given anxiolytic drugs (reviewed in Maestripieri et al. 1992). However, a major difference between the measure of cortisol and self-directed behaviors in this study is that fGCMs represent a global measure of cortisol response of an individual over an approximately 24 hour period, where self-directed behaviors are generally reflective of anxiety related experiences only in the previous few minutes (e.g. Aureli, 1997, Judge & Mullen, 2005). Therefore, the global measures represented in fGCMs are better able to catch seasonal changes, where rates of self-directed behaviors are more specific to only the observation period.

As mentioned above, the formation of social bonds is also critical during the adolescent period due to their potential long-term health benefits. As predicted, grooming rates significantly increased from the juvenile to adolescent period, however, they did so only with adult females. This pattern is to be expected as adult females represent the most stable social partners in rhesus macaque society. Adult females remain in the natal group for life, and therefore established bonds will last for life. In contrast, adult males can transfer between groups and offer no guarantee of a stable, long-term social bonds. More interestingly, grooming was also influenced by the female sex hormone progesterone. The positive association between grooming behavior and progesterone is similar to results in the human literature, suggesting that progesterone plays a similar role in social affiliation in many primates, not just humans. For example, Taylor et al.

(2000) have suggested that, in females, the classic “flight-or-flight” stress response may actually be better described as a “tend-and-befriend” response. The theory of tend-and-befriend posits that in response to stressors females may be able to draw behavioral responses from the attachment-caregiver system in order to both enact nurturing behaviors that can protect the self and/or offspring (“tending”) as well as create social bonds that may help that aid the nurturing process (“befriending”). While the majority of work investigating the underlying mechanisms of tend-and-befriend have focused on other physiological markers, particularly oxytocin (e.g. Taylor 2006) and endogenous opioids (e.g. Taylor & Master 2011), the data presented here offer an additional biomarker for consideration when investigating the tend-and-befriend stress response of females. This study also suggests that female rhesus macaques may make a good model species for testing the tend-and-befriend hypothesis, which currently has only been directly tested in a very limited number of studies in nonhuman primates (e.g., spider monkeys, Rodrigues 2013, marmosets, Cayanaugh et al. 2016), none of which have looked at the role of progesterone in relation to stress responses or affiliative behavior. Future work in this species should focus on trying to capture more fine scale measures of progesterone concentrations (e.g. via blood or saliva) taken immediately prior to or following grooming bouts. Further, work with captive animals would allow for the injection or blockage of progesterone, and a causal mechanism between grooming and progesterone could be more clearly established.

CHAPTER 4

The function of sexual swellings in adolescent female rhesus macaques

ABSTRACT

Exaggerated swellings of the anogenital region are common among female Old World monkeys and some apes. These signals may give a probabilistic cue of female fertility (the “graded signal” hypothesis) or indicate some information about the quality of a female (the “female quality” hypothesis). In rhesus macaques (*Macaca mulatta*), only adolescent females display a sexual swelling in their initial mating season, but fail to display the swelling in any subsequent mating seasons. I investigated the function of these swellings to see if they conformed to one of the two hypotheses proposed in other species. In this species, swellings do not seem to give a cue of ovulation, nor do they seem to indicate some measure of female quality. These results indicate that the current framework explaining the function of sexual swellings in catarrhine primates is insufficient to explain the existence of the trait in rhesus macaques, and that more work is needed to uncover the exact function of the trait.

INTRODUCTION

Exaggerated anogenital swellings in females are common among the Old World monkeys and some apes. These swellings are known to be estrogen dependent, can get quite large and colorful, and tend follow a similar pattern: increases in swelling/reddening during the follicular phase, peak swell/redness in the periovulatory period, and a detumescence/lessening of redness during the luteal phase of the menstrual cycle. Much research has been dedicated to investigating the role that these signals play. Indeed, Darwin (1876) himself noted that these signals probably serve, “as a sexual ornament and attraction.” Despite their commonality, these swellings are not universal among the catarrhines, suggesting that the trait has evolved independently several times (Dixson 1983).

Two main hypotheses have been proposed to explain the role of “sexual swellings.” The first, proposed by Nunn (1999), is called the graded signal hypothesis. This hypothesis suggests that swellings provide an approximate, rather than exact, cue regarding female fertility. The graded signal hypothesis acknowledges the limit of the signal, however, by the fact that that peak size gives only an approximation for female fertility as swellings tend to become maximally swollen for several days around the time of peri-ovulatory period. The occurrence of ovulation within the maximum swelling period varies between species. In mandrills, lower ranked females have much longer and larger swellings than higher ranking females. In this species, males invest a large proportion of time and energy mate guarding the females during their times of swelling. However, they begin and invest in mate guarding well before conception likelihood, indicating that males are unable to use the swellings to exactly pinpoint the time of ovulation (Dixson 2012). Chimpanzee females also have exaggerated swellings. Deschner et al. (2004) investigated both the influence of the swelling size in females, and the reactionary male behavior. The authors found that swellings did peak around the time ovulation, though did not time it exactly. Further, when high ranking males have to choose between several females displaying the signal simultaneously, they tend to prefer females in the fertile phase of the cycle rather than just the female with the biggest swelling. Therefore, across most species, although swellings are used by males to allocate their mating efforts, it is unlikely that they provide exact cues of ovulation.

The second hypothesis suggests that sexual swellings in females have evolved to in response to intra-sexual selection among females, and these swellings serve as an indicator of female quality. For example, Domb and Pagel (2001) found that in olive baboons (*Papio hamadryas anubis*), female swelling size was positively correlated with a younger age at first conception, number of offspring born per year, and number of surviving offspring per year. Further, male baboons were more interested in females with larger swellings, fought more often with consorts of females with large swellings, and were more persistent in following already guarded females with larger swellings compared to those with smaller swellings. The authors argue that swellings are, "almost certainly costly to produce" (although the authors did not measure any type of costs in their own subjects) and therefore represent an honest signal of female reproductive quality and health. However, Zinner et al. (2002) conducted a reanalysis of Domb and Pagel's (2001) data and found several discrepancies. For example, they report that the female height explained a large portion of the variation in female swelling size. Further, the data initially presented were pooled from five different study groups, but the groups showed significant variation in swelling length, age at first conception, and yearly number of surviving offspring. Further, the groups differed in food availability and adult sex ratio, both of which can alter female reproductive success. Therefore, while the indicator of female quality hypothesis is theoretically plausible, there is little empirical evidence to support it.

In their investigation of swellings among catarrhines, Alberts and Fitzpatrick (2012) note that, whatever the function of sexual swellings (e.g., graded signal, quality indicator), they can vary in one of three ways: among a single ovulatory cycle within a single female, between multiple ovulatory cycles within a single female, or between females. These variations may be different between different species, suggesting that the functions of swelling may be different in different species. A review by Anderson and Bierlert (1994) notes that there are several catarrhine species in which adolescent females display an exaggerated version of adult signals (e.g., geladas, anubis baboons, gorillas). They hypothesized several possible functions of the exaggerated traits including a barrier to cross-species hybridization, an aid in female-female competition, an aid for transfer between groups, an additional signal to assist when circumstances of local ecology are likely to be unpredictable, or a nonadaptive side-effect of other phenomenon.

In rhesus macaques (*Macaca mulatta*), adolescent females show exaggerated sexual swellings but adult females don’t. Both adolescent and adult females, however, possess another type of graded signal, which may or may not be functionally related to sexual swellings: facial redness. Female facial redness varies significantly throughout the ovarian cycle and peaks during the fertile phase (Dubuc et al. 2009). This peak in facial redness is enough to convey to males a probabilistic cue of ovulation, though only males who are familiar with a female are able to read this cue (Higham et al. 2011). While the role of red coloration in the face and anogenital region has been fairly well investigated in rhesus macaques (Waitt et al. 2006, Dubuc et al. 2009, Higham et al. 2011), to date little research has investigated the role of swellings as a potential signal in adolescent females. Anogenital swelling in rhesus macaques is not merely an “exaggerated” version of an adult trait (as reviewed by Anderson and Bierlert 1994), but altogether a separate potential signal. Therefore, the goal of this research was to investigate the function of these swellings in a specific age class that fails to display in later life stages.

If the graded signal hypothesis is operating in the rhesus macaques, I predicted that swelling size would be greatest in weeks where females were cycling and lower in non-cycling weeks. If the female quality hypothesis is operating in rhesus macaques, I predicted that males would engage in more courtship behavior (e.g., muzzle-ups, grooming) towards females with larger swellings than with those with smaller swellings, other things being equal (e.g. the stage of their cycle or their age or dominance rank).

METHODS

Study Site and Subjects

This study was conducted on Cayo Santiago, a 15.2ha island located 1 km off the southeast coast of Puerto Rico. A rhesus macaque colony was established in 1938 with wild individuals captured in India (Rawlins and Kessler 1986). All animals are habituated to human presence. At the time of the study, the population on Cayo Santiago was approximately 1400 individuals separated into eight naturally formed social groups. The subjects of this study were 22 females born between August-September of 2011 from two of the eight social groups. The subjects were approximately equally distributed across dominance ranks (high: N = 5, middle: N = 8, low: N = 9).

Swelling Assessment

In most primates that display sexual swellings (e.g., baboons, chimpanzees), the swelling is confined to the anogenital region. However, some species are different, such as the long-tailed macaque (*Macaca fascicularis*), where the swelling is concentrated to the base of the tail (Engelhardt et al. 2005). In rhesus macaque adolescents, the swelling can be found in both places, as well as down the back of the legs (see Figure 4.1). In order to assess the variation in swelling size among the females, subjects were rated on a 4-point scale adapted from Dixson (2009). The definitions of the points are listed in Table 4.1. Each subject was rated independently by three researchers over the same 24-hour period, and the three scores are averaged to give each female a weekly swelling score.

Figure 4.1. Example of a maximum swelling size in a female rhesus macaque.

Behavioral Observations

Behavioral data were collected five days a week from 0700-1430 hours via focal animal sampling (Altmann 1974). Each animal was observed for 30 minutes once a week. Focal follows were counterbalanced bi-weekly between morning (0700-1030 hours) and afternoon (1030 to 1430 hours) sessions to control for diurnal effects on behavior. The types of behavior collected include affiliative (e.g., grooming), agonistic (e.g., threats, submission), sexual (e.g., copulation, solicitation), and self-directed (e.g., scratching, yawning). Appendix 1 contains the full ethogram with all behavioral descriptions.

For interactive behaviors (e.g., grooming) individual ID of the partner was recorded if possible, otherwise the age-sex class of the interactant was used (e.g., juvenile male, adult female). Further, the directionality of all behaviors was recorded (e.g., whether the subject was grooming or being groomed). For non-interactive behaviors, the nearest neighbor within 1m is recorded. All data were recorded using the Behaviour software on a Psion Workabout. Data were parsed into an Access database (Microsoft Corp., Redmond, WA, USA) and queries were used to obtain frequency and duration of behaviors.

In addition to focal animal follows, all subjects were surveyed four times daily to assess estrus status. Females were considered to be in behavioral estrus if they were seen mating, soliciting males, or if they had a sperm plug.

Table 4.1. Swelling score descriptions.

Swelling Score	**Definition**
0	No signs of any folding, swelling, or wrinkling of the skin in anogenital region
1	Wrinkling or folding of the skin around the anogenital region
2	Some swelling (but not fully tumescent) of the skin in the anogenital area, tail base, and/or back of legs
3	Fully tumescent swelling of the skin in the anogenital area, tail base, and/or back of legs

Assessment of Early Life Adversity

All subjects were part of a larger dataset for a previous project in the Behavioral Biology Laboratory which collected behavioral data on mother-infant interactions for the first 12 weeks of life. In these first 12 weeks, the subjects were video recorded for 30 minutes twice a week. Videos were coded for mean hourly rates of maternal rejection and abuse (see ethogram). Abuse and rejection rates were averaged across the 12-week study period to obtain a single value for overall early life adversity (ELA), and subjects were binned into 3 categories: high, moderate, and low. These categories have been shown to be behaviorally and physiologically valid (see Mandalaywala et al. 2014).

Fecal sample collection and hormone assessment

All subjects had opportunistic fecal samples collected from them every 2-3 days in order to assess concentrations of the sex hormones estrogen and progesterone. Previous work in this population has showed that fecal samples collected from the same individual in the morning and afternoon show no differences in hormone concentrations (Rahkovskaya & Heistermann, n.d.). A total of 462 fecal samples were collected from all 22 subjects. On average each female had 21.0 ± 0.81 samples collected across the 15 week study period, or 1.4 ± 0.05 samples per individual per week.

Fecal samples were collected only after defecation was observed directly and the sample was uncontaminated with urine. Samples were subsequently homogenized, removing any nonfecal debris, and a small bolus of approximately 1.0g was placed in a 20ml tube. These tubes were stored in insulated coolers containing ice packs. This method has been validated in the field as reliably storing samples until they can be brought into a freezer without altering hormone concentrations (Hodges & Heistermann, 2011). Upon returning from the field, fecal samples were frozen at -20°C until they were shipped on dry ice to the Primate Reproductive Ecology and Evolution lab at NYU. All samples arrived frozen. Fecal samples were prepared for Enzyme-Immuno Assay (EIA) by being lyophilized and pulverized followed by extraction of an aliquot (50-70 mg) of the fecal powder with 3 ml 80% methanol by vortexing for 15 min (Higham et al., 2009). After fecal extractions were performed, the samples were shipped to the German Primate Center at the University of Göttingen, where the EIAs were carried out.

Progesterone and estrogen concentrations were measured by pregnanediol-3-glucuronide (iPdG) and total estrogens (iE_{total}) metabolites respectively, both of which have been validated for use in macaques (Engelhardt et al. 2004). Intra- and interassay coefficients of variation, determined by replicates of high and low value quality controls. Progesterone metabolite interassay variation had values between 4.6% (high) and 6.9% (low), and intra-assay values between 4.1% (high) and 6.2% (low). Estrogen metabolite interassay variation had values between 10.9% (high) and 8.8% (low), and intra-assay values between 3.0% (high) and 6.3% (low). All assays are standardized for differences in fecal weight and are expressed as concentration (ng) per dry fecal weigh (g). Hormone concentrations were positively skewed, so to achieve normal distribution, hormone concentrations were log transformed for all analyses.

Data Analyses

In order to characterize swellings across females, both the maximum swelling size a female reached during the mating season as well as her average swelling size for the entire mating season were compared between the different ranks (high, middle, and low) and categories of early life stress experiences (high, moderate, and low) using the non-parametric Kruskal-Wallis test.

In order to assess if swelling size relayed information about cycling/ovulation (i.e., the graded signal hypothesis), swelling size was compared between weeks in which a female was in estrus and weeks in which when she was not. A Linear Mixed Model (LMM) used with swelling size as the dependent variable, estrus status as a fixed factor, and individual ID as a random factor to control for repeated observation. In order to assess if swelling size relayed information about female quality, weekly measures of both male courtship behavior (i.e., muzzle ups and grooming) and female proceptivity (sexual presentations) were analyzed in relation to weekly swelling size.

RESULTS

Swellings and hormones

Neither maximum swelling size (Kruskal-Wallis $X^2 = 1.622$, $p = 0.444$) nor average swelling size across the season ($X^2 = 0.901$, $p = 0.637$) was related to dominance rank. Similarly, neither maximum swelling size ($X^2 = 0.215$, $p = 0.898$) nor average swelling size across the season ($X^2 = 1.783$, $p = 0.410$) was related to early life stressful experiences.

Sexual swellings did not show a significant relation directly to either sex hormone. Neither PdG ($F_{31,161.84} = 0.755$, $p = 0.820$) nor E1C ($F_{151,38.55} = 0.967$, $p = 0.573$) were directly related to changes in the swelling size. Further, the ratio of E1C to PdG (E/P ratio) was also not significantly related to changes in swelling size ($F_{197,8} = 0.956$, $p = 0.597$). However, swelling sizes did decrease significantly after a female became pregnant ($F_{2,319.29} = 12.281$, $p < 0.001$, Figure 4.2). Therefore, pregnancy status was controlled for in all analyses.

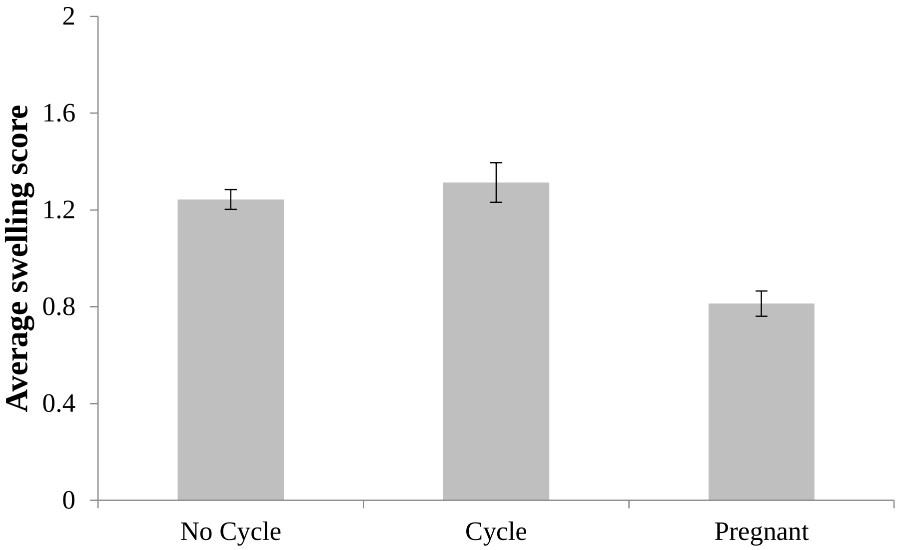

Figure 4.2. The average swelling scores (± SEM) for females in weeks when they were not in estrus (1.243 ± 0.041), were in estrus (1.313 ± 0.082), or were pregnant (0.813 ± 0.052).

Graded Signal and Female Quality Hypotheses

Sexual swelling size did not differ between weeks in which a female cycled and weeks in which she did not ($F_{1,210.55} = 2.362$, $p = 0.126$, see Figure 3.3). Increased sexual swellings do not seem to indicate a probabilistic cue of ovulation. Furthermore, increases in sexual swelling size did not make a female more sexually attractive to males. Male muzzle-ups were not more common towards females with increased swellings ($F_{23,181.45} = 1.234$, $p = 0.221$). Additionally, copulation rates did not vary with swelling size ($F_{18,187.81} = 1.369$, $p = 0.151$). Further, female soliciting behavior towards males did not vary with changes in swelling size ($F_{22,183.57} = 1.258$, $p = 0.205$). Finally, grooming rates were not influenced by swelling size ($F_{6,203.20} = 1.024$, $p = 0.411$). Increased sexual swellings do not seem to indicate female quality.

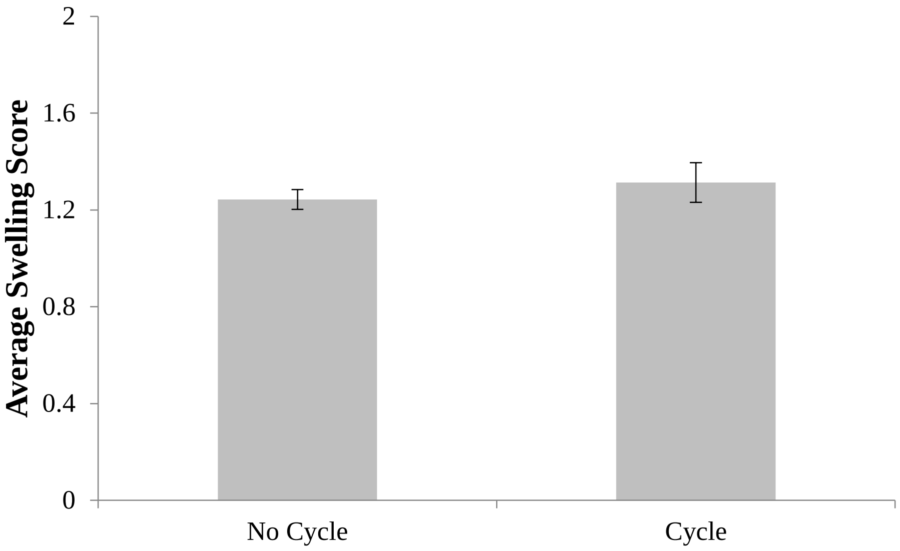

Figure 4.3. The average swelling scores (± SEM) for females in weeks when they were (1.313 ± 0.082) and were not (1.243 ± 0.041) in estrus.

DISCUSSION

Based on the data in the current study, the function of sexual skin swelling in adolescent rhesus macaque females remains unclear. The swelling data do not fit with the predictions of the graded signal hypothesis, as the swellings are not larger during weeks when the females were in estrus. Further, overall the swellings did not fluctuate cyclically in size as regularly as is observed in other species. In most of the subjects, the swellings either generally increased or decreased over the mating season.

Similar to other species, adolescent rhesus macaques are not highly desired as mating partners. They have a much higher rate of infant mortality and are less likely to carry a pregnancy to full term. Therefore, it is possible that the function of this signal is to allow females to entice reluctant males into considering them as mating partners. For example, in chacma baboons, ovariectomized females fitted with artificial swellings (i.e., female sexual behavior was eliminated but the visual signal was present) significantly increased male sexual interest as measured by seminal emissions (Girolami and Bielert 1987). However, the swellings in adolescent rhesus macaques seem to fail to elicit increased interest from males, since increases in swelling size did not increase the rates of grooming, muzzle ups, or copulations with adult males.

Most interestingly, swelling size changes in adolescent rhesus macaques did not correlate directly with the female sex hormones, which is the case for most other species (e.g., *Macaca nemestrina*, Carlisle et al. 1981; *Pan troglodytes*, Ozaza and Gould, 1982). However, given the significant decrease in swelling size after conception, hormones are clearly involved with the swellings in rhesus macaques. Dixson and Anderson (2004) posit that in some species the sexual swelling serves a secondary function related to cryptic female choice. For example, in chimpanzees a female's swelling can increase vaginal depth by 50%. This induces sperm competition in males as those with genitals who can overcome the greatly increased size would outcompete those who cannot. This does not seem to be the case in rhesus macaques for two reasons: 1. the swelling only occurs in adolescents, and 2. the size of the swelling does not greatly increase vaginal depth to the extent seen in chimpanzees.

In their review, Dixson and Anderson (2004) also point out that females may be differentially reactive to circulating hormone levels. Given that adolescence is a period of reactivation of the Hypothalamic-Pituitary-Gonadal (HPG) axis, females may simply have over- or under-reactive response to the newly circulating estrogens upon the resurgence of the HPG axis at puberty. Future work in this species should focus on collecting tissue samples from the swellings to see if estrogen receptor densities or sensitivities are different between different females. Further, studies comparing the swelling sizes of related females, such as mothers and daughters or full or half-sisters, could also help elucidate if there is a genetic component to estrogen sensitivity that is manifested in adolescence. Overall, the results of the study call in to question the current framework surrounding the function of sexual swellings in catarrhine primates. Adolescent rhesus macaques clearly display this visual signal, yet its function does not fit with the two leading theories to explain their function. We therefore must include an additional consideration when explaining this visual cue among primates. Future work must also consider why this signal only occurs in adolescent individuals and fails to reoccur in adults.

CHAPTER 5

The development of red facial bias in rhesus macaques

ABSTRACT

Sexual selection has driven the evolution of a variety of exaggerated traits in both males and females that can serve as a tool in attracting mates of the opposite sex (intersexual selection) or as a means of outcompeting members of the same sex (intrasexual selection). In rhesus macaques, male facial redness has been shown to be a trait that is independent of dominance rank, but nonetheless is highly attractive to females. Previous work in this species has shown that both adult females and, to a degree, adult males have a preference for looking at darker red faces, but young juveniles do not distinguish facial color in a looking time paradigm. The purpose of this study was to determine where in development this bias for looking at darker red faces develops. Unlike previous studies (Dubuc et al. 2016), this study found no bias in looking time for any age-sex class that was tested (i.e., males and females between 2 and 4 years of age). More work is needed to determine how exactly adults of this species develop a bias towards looking at darker red faces in males.

INTRODUCTION

Across the animal kingdom, sexual selection has driven the evolution of a variety of exaggerated traits in both males and females. These exaggerated traits can serve as signals of individual quality to attract mates (intersexual selection), or to signal competitive ability to same sex conspecifics (intrasexual selection). These signals vary widely, such as the attractive ornamentation in the plumage of birds (Price 1998), or the large horned weapons of cervids

(Clutton-Brock 1982). Primates as a taxonomic group have evolved a wide variety of sexually selected traits for both intra- and inter-sexual competitive purposes. For example, many species have evolved large canine teeth and/or increased body mass to aid in direct male-male competition (Plavcan et al. 1995).

Primates are also a highly visual taxonomic group, particularly compared to many other mammalian orders. Unique to the catarrhine primates is trichromacy, the ability to see in the red part of the visual spectrum (Surridage et al. 2003). The trait originally evolved in response to foraging pressures in order to distinguish ripe fruit, flowers, and/or young leaves against a darker green background of foliage (Dominy & Lucas 2001). It is unsurprising, then, that many catarrhine primate species have developed highly colorful visual signals to convey information to both potential mates as well as same sex competitors. For example, male vervet monkeys (*Cercopithecus aethiops*) have blue colored scrota, the brightness of which conveys the social status of the male to other individuals (Gerald 2001). Both mandrills (*Mandrillus sphinx*) and drills (*Mandrillus leucophaeus*) have bright red facial coloration, which changes rapidly in response to current dominance rank in the social group (Setchell and Wickings 2005; Marty et al. 2009). Further, in mandrills dominance rank, and therefore facial redness, is heavily correlated with reproductive success. Similarly, male geladas (*Theropithacus gelada*) display a red chest patch, which is darker in harem leaders (as opposed to non-reproducing follower males). Further, when comparing the redness of only leader males, males with larger harems (i.e., harems with more than 6 females) had darker chest patches than leader males with fewer females in their harems (Bergman et al. 2009).

In the above examples, the red ornamentation has been mostly studied from the perspective of intrasexual selection, whereby the color of the ornaments signals to same sex conspecifics information about the dominance rank or social status of the male in relation to other males. The trait could also arguably be applied to intersexual competition, whereby females are choosing mates based on the quality of the ornament. However, in many of the species in which the traits occur and have been studied, dominance rank is highly correlated with reproductive success, with the reproductive skew heavily favoring higher ranked individuals. Red skin ornamentation is expressed due to fluctuations in blood flow to the epidermis, which is controlled by sex hormones. Specifically in males, testosterone is aromatized into estrogens in the skin, which then activate the estrogen receptors which cause the skin to display the color (Rhodes et al. 1997). The potentially dual role of the signal (i.e., as both an inter- and instrasexual signal) is due to the fact that this biological process can inform about both the underlying physiological condition of the male, as well as his competitive ability. Physiologically, the signaling process relies on both the flow and oxygenation of the blood. Further, androgenic hormones, namely testosterone, have been implicated as a "double edged-sword," because while they enhance dominance and status signals (such as redness, aggression, etc.), they can also act as an immunosuppressant (e.g., Wichmann et al. 1996). Therefore, according to the Handicap principle, only those individuals in the best bodily condition can afford to display the most intense red traits, since they can afford to maintain the necessary levels of immunosuppressing testosterone to gain the darkest red displays (Zahavi 1975, Folstad and Karter 1992).

An interesting case to study the role of red skin signals, where it is possible to disentangle dominance rank or social status from reproductive success, comes from rhesus macaques (*Macaca mulatta*). In this species, adult males have a red facial display, but unlike many of the other species mentioned above, this signal does not seem to be related to dominance rank

(Higham et al. 2013, Dubuc et al. 2014a). Further, females of this species have a great degree of freedom in mate choice (Manson 1992), and seem to choose males based on degree of facial redness, where they prefer darker red males. For example, Dubuc et al. (2014a) found that females actively try to solicit darker red males for sex, and this increased attention towards darker males results in higher reproductive success in these males (Dubuc et al. 2014b). In the Cayo Santiago rhesus macaque population, there is very little reproductive skew in rhesus macaques based on dominance rank, as in this population dominance rank is not based on competitive ability or direct competition, but follows a queueing pattern where males enter a new group at the bottom of the hierarchy and move up in rank with greater tenure length in the group (Berard 1999).

Previous work with the rhesus macaques on Cayo Santiago by Dubuc et al. (2016) investigated the role of the red facial signal of adult males. Specifically, they employed a looking time paradigm to see if adult males and/or adult females have a bias towards looking at darker red faces. They also used juvenile individuals (~16 months old) as a control group, to check to see that if both groups of adults had the preference for looking at dark red faces, and if this bias was present from birth. Their results indicated that both adult males and adult females showed a bias in the overall looking time, where they spent significantly longer looking at the darker red faces. However, when looking at the *proportion* of individuals who demonstrated a bias towards the dark red face (independent of how much of a difference in looking time there was), only adult females showed a significant bias towards the dark red faces. The failure to find a male bias in this proportion metric, when finding it in the looking time, is explained by the fact that some males find the dark red faces extremely salient, while others do not, so the overall looking time difference is driven by just a portion of the males. The authors suggest that males may be using more cues to assess other males than just their facial color, such as facial musculature. They find this suggestion probable because males had a greater overall looking time towards both faces compared with females, suggesting they are perhaps assessing multiple cues (looking time males: 3.77 ± 0.22 s; females: 2.75 ± 0.15 s).

Interestingly, juvenile individuals did not display a bias towards looking at dark red faces in either metric. This is not due to disinterest in looking at faces, however, because juvenile individuals actually had greater overall looking time towards both faces, compared to adults (looking time juveniles: 4.59 ± 0.24 s; adults: 2.49 ± 0.14 s). Therefore, this preference towards looking at dark red faces is not some intuitive bias that all individuals are born with, despite the color red being a salient cue for this species across contexts (Hughes et al. 2015).

The purpose of this study was to determine how the preference for looking at dark red faces develops, building off the previous work of Dubuc et al. (2016) who showed that 16 month old juveniles do not prefer looking at the red facial cue, but that adult females, and some adult males, do. I tested both male and female individuals in the 2 year, 3 year, and 4 year old cohorts. These individuals represent a slight overlap with Dubuc et al.’s (2016) test range as they defined adult as any individuals greater than or equal to 3 years of age. Therefore, while they did test some individuals 3 and 4 years of age, their range of adults ranged from 3-18 years old in females and 3-23 years old in males. Therefore, by specifically looking at the immediate age-sex classes in between the test groups in their study, I hope to elucidate a clear picture of how and when the red facial signal becomes salient to certain individuals. One possibility is that this signal is learned in an age dependent manner, such that after experiencing a certain number of seasons where the signal is present, all individuals of a certain age class (and possibly sex) will learn to focus on it. If individuals learn the saliency of this signal due to mating experience, only the older (i.e., 4 year old) females in this sample have had at least one season of experience as active participants in the mating season. Further, depending on when in the season individual 3 year olds are tested, they may or may not also have some experience mating, and would therefore display a mixed result where some individuals find the signal salient while the unexperienced females would not. Males were also tested because of the fact that at least some of the males in previous studies showed a strong preference for looking towards the dark red faces, but the authors did not elucidate which subset of males was driving the overall difference in looking time. If it is younger males, this study may help clarify that.

METHODS

Study Site and Subject Selection

This study was conducted on Cayo Santiago, a 15.2ha island located 1 km off the southeast coast of Puerto Rico. A rhesus macaque colony was established in 1938 with wild individuals captured in India (Rawlins and Kessler 1986). All animals are habituated to human presence. At the time of the study, the population on Cayo Santiago was approximately 1400 individuals separated into eight naturally formed social groups.

The experiments were conducted during the peak of the 2015 mating season, between February 19 and April 23. By testing the animals during the peak of the mating season it allowed examination of the effect of the bias during the time of year when males are actually displaying this trait and reducing any bias toward the signal due to a novelty effect or any violations of expectation because of incongruence (i.e., subjects might look more at red faces in the birth season simply due to the fact that red faces are not typically present during the birth season and not because they have a preference for looking at them). A total of 6 age-sex categories were tested, the total counts for each are described in Table 4.1. The subjects sampled were from two of the eight social groups (R and S). The age categories were selected because previous research has demonstrated that young juveniles (approximately 16 months of age) do not show a bias for looking at dark faces, but adult individuals do (Dubuc et al. 2016). In Dubuc et al.'s study, their definition of adult was any individual >3 years of age. This resulted in a wide range of ages for their subjects (3 to 18 years of age in females; 3 to 23 years of age in males). Therefore, the best way to determine where the developmental shift in the emergence of this bias occurs it was necessary to select individuals in the ages between the two groups previously tested.

Table 5.1. Number of subjects in each age-sex class.

Age (years)	**Males**	**Females**	**Total**
2	19	26	45
3	27	26	53
4	14	18	32
Total	60	70	130

Collection and Preparation of Facial Stimuli

A set of stimuli were created from photographs of 5 male rhesus macaques collected in group V, which was chosen due to limited interaction group V has with the groups that were tested (groups R and S), and therefore all subjects are very unlikely to have any experience or familiarity with the individuals used in the stimuli. The images were created using the same methodology as Dubuc et al. (2016) but are described in full here. The images were manipulated to display either a dark red or pale pink color in the face (see Figure 4.1). The color of the manipulated images were generated based on photographs taken of 24 different males at the peak of a previous (2012) mating season, for a study conducted by Dubuc et al. (2014). In order to assess average facial color, images were captured in RAW format and a color standard was used for standardization of the images due to changes in light and luminance. Skin color and skin darkness were measured as the average of red (R), green (G), and blue (B) over a fixed, standardized portion of the face. The R, G, and B values are then transformed from the camera's color space to rhesus macaque color space, a method that has been validated by Stevens et al. (2009). This method provides estimates of short-, medium-, and long-wavelength photoreceptor catches of the rhesus macaque visual spectrum. The 24 original males were photographed over several days and each male had his average color calculated.

Once the facial color averages were calculated, principal components analysis was used to determine the most important dimension of the variation in facial color. The pale pink and dark red color manipulations were then selected as 2 standard deviations above and below the average facial color, respectively. These manipulated colors were then applied to the 5 images of the males from group V, which were selected because they were oriented toward the camera without looking directly at it, and were displaying a neutral expression. All stimuli used were free from any obvious or distracting facial features that might divert a subject's attention, such as scars or fresh wounds. Each of the 5 male images had both a pale pink and dark red manipulation prepared. Images were created using the image-editing tools in GIMP 2.0. Faces were selected with the intelligent scissors tool, and then the color alteration was performed using the Hue Saturation tool. In order to create natural looking stimuli, the airbrush and eraser tools were used around features like the eyes and hairline. The background of the images were also altered to appear to have the same blurred foliage in the background. Photos were printed on matte photo paper (Staples Photo Surpeme) using a Canon Pixma Pro 100 color-calibrated printer. The faces were all then measured with a Xrite ColorMunki spectrophotometer to check the printed colors were the same as the target colors, that is, as they were intended to be printed in the rhesus color vision spectrum.

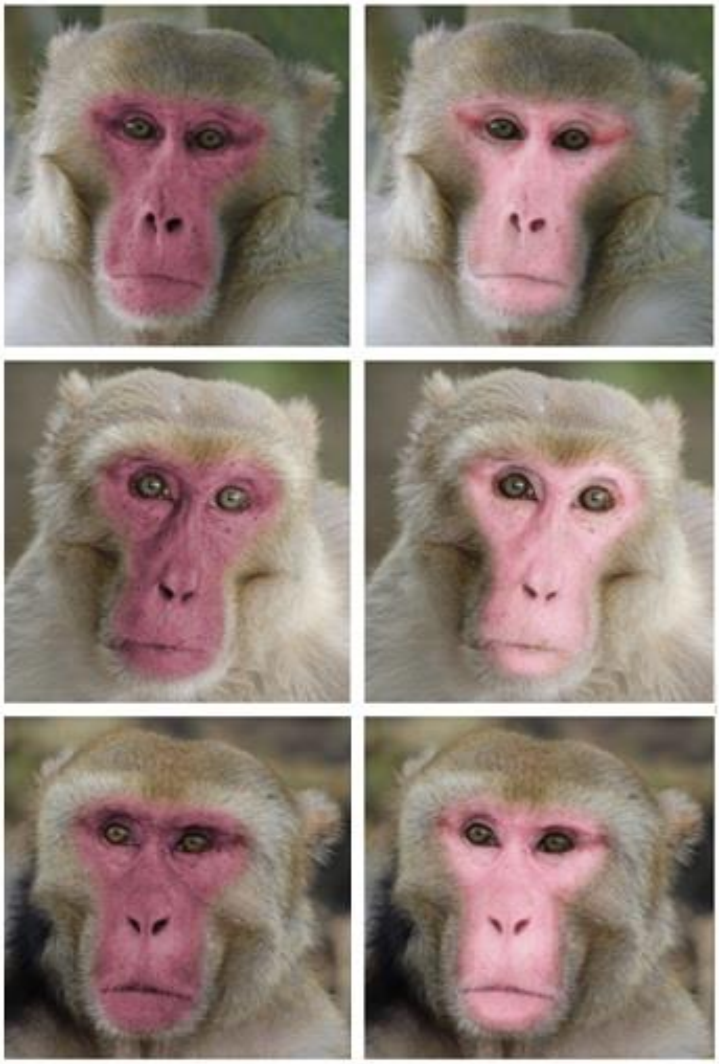

Figure 5.1. Three examples of the same male face manipulated to be dark red (left) or pale pink (right). Photographs by Constance Dubuc.

Apparatus and Experimental Procedure

Subjects were presented with 2 simultaneous pictures depicting 2 different male faces that were differently colored (i.e., one dark colored face and a different light colored face). These pictures were inserted into frames that measured 1 m apart from the center of each photo, and 78.5 cm apart from the inside frame of each photo. The images were hidden with blue occluders that slide in and out of the frames. The identity of each image, the color of the image, and the side of each image (i.e., whether the dark image was on the left or right side) were randomly determined prior to the onset of the experiment. Each test session was performed by two experimenters: one to operate the testing apparatus, and the other to film the subject.

To begin a test session, the experimenters would first identify a potential subject who fit one of the age categories, was slightly socially apart from others in the group, was in a resting or otherwise inactive state, and had open testing space in front of them. One experimenter would then set up the apparatus approximately 1-3 m away from the subject, on level ground, in similar lighting conditions. The experimenter also ensured that both images were equidistant from the subject and that there was nothing occluding the apparatus (e.g., rocks, shrubs). Once the apparatus was set up and the experimenter was settled in the center of the apparatus, the camera operator began filming. The experimenter at the apparatus would then direct the subjects attention to each of the stimuli, then to the center of the apparatus and remove the occluders, all while having their face oriented down, thus preventing any interaction or influence with the subject. The subjects looking behavior was recorded for a maximum of 20 s. The camera operator was blind to all experimental conditions, and was responsible for deciding to abort a test session. Test sessions were aborted if the subject did not pay attention to the apparatus, if the subject moved out of the range of the camera, or the subject was distracted by something else (e.g., a conspecific approach).

Video Coding

Each trial was coded on a frame-by-frame basis (30 frames/s) by trained coders blind to experimental conditions using QuickTimePlayer (httpss://support.apple.com/downloads/

quicktime). Coders recorded the exact frames at which subjects began and ended looking at each stimulus, or at neither image. Cohen's Kappa was used to assess inter-coder reliability, and the two coders had a Cohen's Kappa score of k = 0.80 agreement with each other, which is considered "substantial" strength of agreement (Landis and Koch 1977).

Statistical Analyses

First I looked to see if there were overall differences in interest at looking at facial stimuli overall, regardless of their color. Therefore, a Mann-Whitney U test was used to test for overall looking time at the faces between the two sexes. Similarly, to test for age related differences in overall looking time, a Kruskal-Wallis test was performed overall looking time at the faces between the three age groups. Finally, to investigate if there were specific age-sex class differences, separate Mann-Whitney U tests between the sexes in age group, and separate Kruskal-Wallis tests were run for testing for differences between the age groups within a sex.

In order to specifically investigate a preference for facial redness, a linear mixed model (LMM) was run. Looking time at each stimuli was set as the outcome variable, but was log transformed to conform to model assumptions. Subject sex (male or female), subject age (2, 3, or 4 years old), and stimulus type (dark face or light face) were set as fixed factors, and subject ID was set as a random factor. To investigate if there were only specific age classes that were showing a facial redness preference, a Wilcoxon signed-ranks test was performed for each age group separately. Further, to investigate differences in specific age and sex classes a Kruskal-Wallis test was performed with each of the 6 age-sex classes using a looking time index. The looking time index is was calculated as follows:

[(time looking at dark stimulus)-(time looking at pale stimulus)] / total looking time where positive values reflect a preference towards the dark stimulus, and negative values reflect a preference for the pale stimulus. Finally, a χ^2 goodness-of-fit test was used to determine if there was a tendency for subjects to look at one face over another. Unlike the previous tests which take into account the degree of how much individuals look at each stimulus, the goodness-of-fit test only determines if a significant proportion of individuals display a preference for looking at one image over the other. The advantage of this test is that it can detect small preferences displayed across many individuals. All analyses were performed in SPSS v23.0. All analyses were two-tailed with a significance level set at alpha = 0.05.

RESULTS

Overall Looking Time

There was not a significant differences between the sexes in overall looking time at the facial stimuli (Mann-Whitney Z = -0.859, p = 0.390), nor was there an overall looking time difference between the different ages (Kruskal-Wallis χ^2 = 0.131, p = 0.936). However, when the sexes are separated by age, 2 year olds show a trend towards significance and 3 year olds significantly differ in looking time, while 4 year olds do not (see Table 5.2). Further, when the sexes are separated, males of different ages were significantly different from one another in overall looking time (Kruskal-Wallis χ^2 = 5.993, p = 0.05; Figure 5.2a), while females were not (Kruskal-Wallis χ^2 = 3.647, p = 0.161; Figure 5.2b).

Table 5.2. Comparisons of male and female combined overall looking time at the facial stimuli, separated by age.

2 Year Olds	**Looking Time (s)**	**SEM**
Male	6.63	0.74
Female	7.90	0.60
Mann-Whitney	Z = -1.884	p = 0.06
3 Year Olds		
Male	7.74	0.57
Female	6.28	0.66
Mann-Whitney	Z = -2.651	p = 0.008
4 Year Olds		
Male	7.04	1.00
Female	8.14	1.32
Mann-Whitney	Z = 0.820	p = 0.837

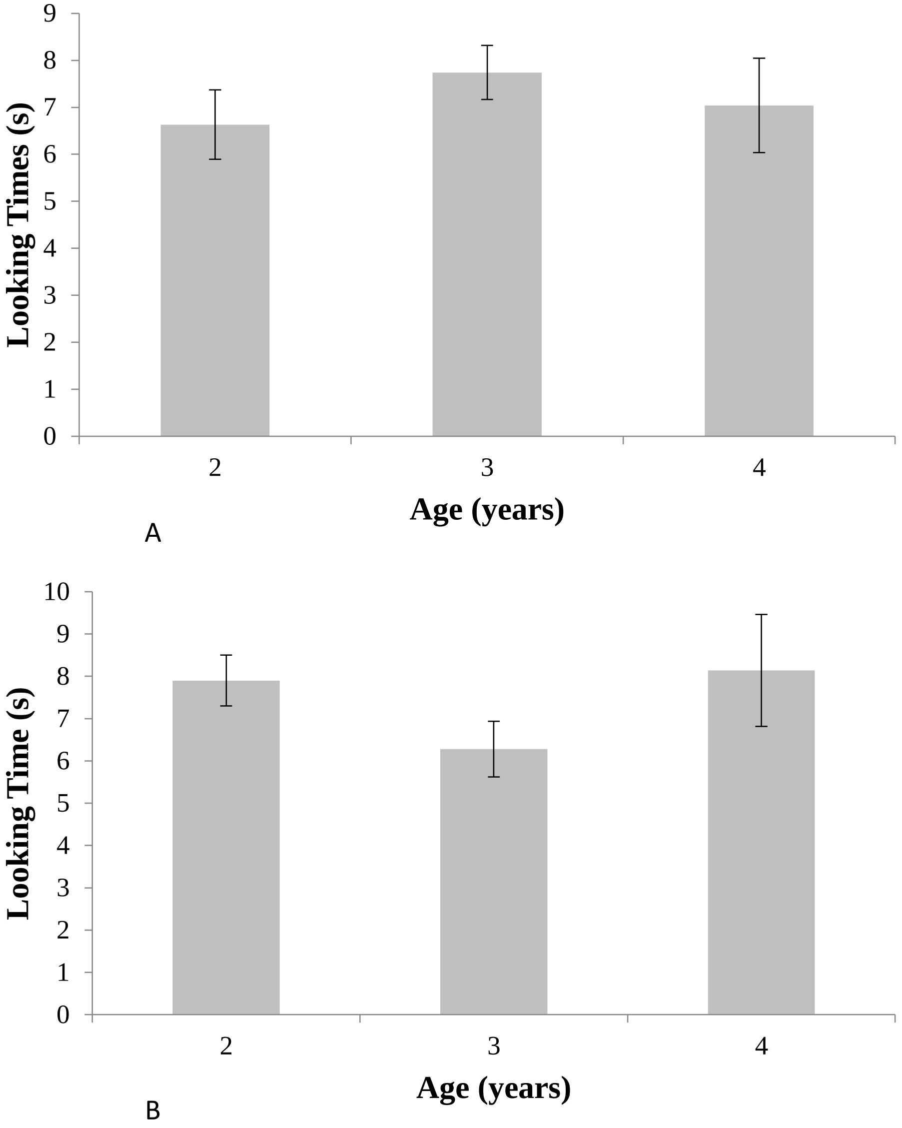

Figure 5.2. Differences in overall looking time at facial stimuli between different age groups, separated by sex, with males in 5.2A (Kruskal-Wallis $\chi^2 = 5.993$, $p = 0.05$) and females in 2B (Kruskal-Wallis $\chi^2 = 3.647$, $p = 0.161$). Error bars represent ± SEM.

Facial Redness Preference

There was not an overall effect of stimulus type on looking time (LMM; $F_{1,141} = 0.510$, $p = 0.477$). Overall subjects had the same looking time for both dark (3.62 ± 0.18 s) and pale (3.68 ± 0.22 s) images. Further, when looking at the individual age or sex classes, there were no significant looking preferences among the 2 year olds (Wilcoxon: $Z = -0.045$, $p = 0.964$), 3 year olds ($Z = -0.623$, $p = 0.533$), or 4 year olds ($Z = -0.327$, $p = 0.743$), nor were there differences between the sexes (females, $Z = -0.768$, $p = 0.442$; males, $Z = -0.472$, $p = 0.637$). When comparing across all 6 of the age sex classes, the Kruskal-Wallis test showed no significant differences between any of the groups (Kruskal-Wallis: $\chi^2 = 5.447$, df = 2, $p = 0.364$; Figure 5.3). Finally, the χ^2 goodness-of-fit test did not reveal significant differences in the proportion of individuals that preferred to look at the dark stimulus compared to the pale stimulus ($\chi^2 = 0.702$, df = 5, $p = 0.982$; see Table 5.3).

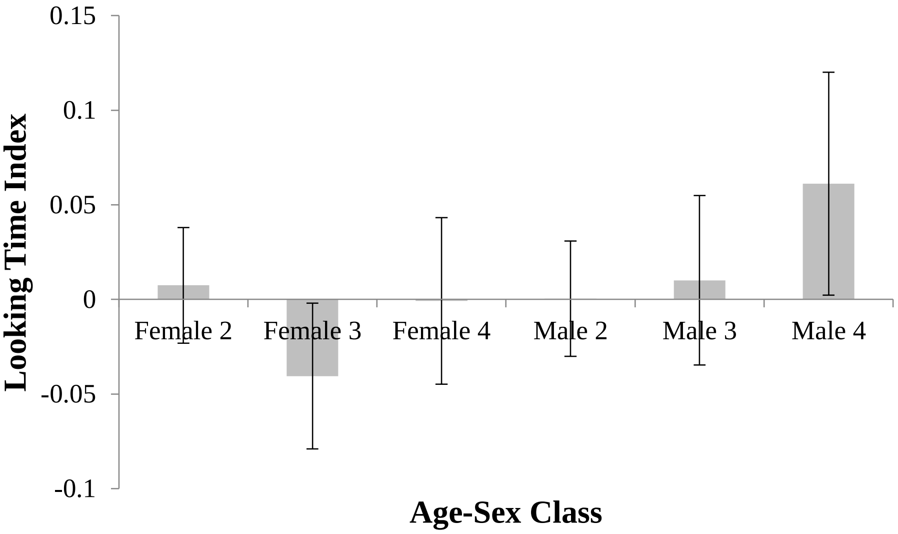

Figure 5.3. Looking time index of the 6 different age-sex classes (Kruskal-Wallis: $\chi^2 = 5.447$, df = 2, $p = 0.364$). Error bars represent ± SEM.

Table 5.3. The number of individuals in each age-sex class and the image they preferred looking at

	Light Stimulus Preference	**Dark Stimulus Preference**
Female 2	13	13
Female 3	13	13
Female 4	10	8
Male 2	8	11
Male 3	13	14
Male 4	7	7

DISCUSSION

Based on the data presented here, the specifics of how and when the preference for looking at dark red faces of adult males remains unknown. Previous evidence suggests that this preference develops somewhere after 16 months of age, but prior to 5 years of age (Dubuc et al. 2016). However, this study specifically investigated the age groups in between those two groups and failed to find any systematic preference among any of the age or sex classes. While this study was based on the work by Dubuc et al. (2016), there are some slight differences which may explain the failure to replicate their findings. For example, in their work they only had three different age-sex classes: adult male (3 years and older), adult female (3 years and older) and juvenile (males and females ~16 months old), while this study had 6 difference age-sex classes, which decreases the number of individuals in each cell in the analyses, which can affect the overall power of the analyses. However, given the high evenness in looking time between the two stimuli types among all the groups in this study, it seems unlikely that statistical power is a major problem here. Also, despite the fact that Dubuc et al.’s (2016) study used all the adults combined, but found similar outcomes if only “young adults” are considered (which they defined as 3-5 years of age for both sexes), they do not separate the results based on the specific ages within those groups. Therefore, given the results presented here combined with those from Dubuc et al., it is entirely possible that their results are being driven by 5 year olds (and older individuals, depending on the analysis).

When considering just the overall looking time at, and therefore interest in, both facial stimuli, again we see differences between previous work and this study. Specifically, Dubuc et al. (2016) report an average of 4.59 ± 0.24 s looking time among their juvenile subjects, while adults looked significantly less overall, an average of 2.49 ± 0.14 s. In this study, the overall average across all ages and sexes was 7.29 + 0.32 s. This further supports the idea that a lack of power is not a problem in the current study, since the subjects were overall very clearly interested in participating in the test, but failing to look at one image more than the other. Indeed, subjects in the current study, on average, were looking almost 3 times as much at the stimuli compared to the adults of Dubuc et al. (2016).

Despite the lack of significant findings in the current study, this still helps the investigation of the role of the red facial signal in adult male rhesus macaques. There is clearly something happening in the 3 to 5 year old age range, given the disparity between the current results and those previously reported. Future studies should therefore take an approach similar to the current study, but expand the age range of those tested, say to 5 or 6 year olds. In this way, it will possible to look specifically to see if these older “young adults” are indeed the individuals driving the results found by Dubuc et al. (2016). Another possible approach would be to employ a longitudinal study which individuals are tested over several mating seasons, to see how their personal sexual history interacts with their looking preferences. For example, it may be possible that females only begin to find the signal salient after a certain number of active mating seasons, or after experience with a certain number of sexual partners. In comparison, a longitudinal study may also help explain why some males find redness extremely salient, while others do not. For example, perhaps only recently emigrated males, or even more specifically only males who recently left their natal group, may use this cue since other males with more tenure in a group may find other cues more salient when evaluating other males.

CHAPTER 6

Conclusions

The studies included in this thesis have furthered our understanding of the factors that influence sexual maturation in female rhesus macaques. Similar to individuals in captivity, dominance rank is an influential factor affecting age at first reproduction in free-ranging rhesus macaques. Additionally, the transition from juvenility to adolescence is physiologically stressful and is accompanied by a suite of changes in social behaviors. Further, while the exact function of adolescent sexual signal production and the development of signal perception have not been fully elucidated, the physiological and behavioral changes adolescents undergo are very likely to play a role in these answers. Overall, these life history traits, which display wide variability in this group, converge with the literature from humans and captive populations, giving a more complete picture of the sources of variation in maturation.

For example, the overall increases in glucocorticoid concentrations from the juvenile to adolescent mating season indicate that, as predicted, navigating a completely new social network is a stressful experience. Female primates transitioning from relying on their mother's protection as an infant to forming their distinct dominance rank as an adult involves much trial and error as they reverse previous dominance relationships (Holekamp & Smale 1991) that were based mostly on size rather than absolute rank. This series of interactions are presumably some of the most stressful times of an individual's life, and I predict that glucocorticoid concentrations would show a reduction once a stable dominance rank is acquired. Further, though outside the scope of this study, I would also predict that in line with studies on other primate species, females who were able to begin forming social bonds with other females would attenuate the stress response that increases during the adolescent period.

The relationship between progesterone levels and female affiliative behavior, while still preliminary, is exciting for the potential of future studies. Only recent work in humans has started to identify this sex hormone with social behaviors, where traditionally researchers have looked elsewhere like oxytocin (Carter 1998) and opioids (Depue & Morrone-Strupinsky 2005). Further, the work in humans so far has only shown associations between the physiology and motivation for affiliation. However, the studies presented here demonstrate that rhesus macaques not only have the same affiliative motivation, but also suggest that this may directly affect behavior as seen by changes in grooming. Because rhesus macaques already serve as a model for human social behavior as well as physiology, these studies offer a new avenue to begin exploring a causal mechanism between progesterone and social behavior in a way that is unavailable in human research. Further, while little work has been done in animal models to test Taylor et al.'s (2000) "tend-and-befriend" hypothesis, stress response behavior and the physiology of the HPA axis is very well studied in rhesus macaques. Therefore, this species is prime for future research of direct investigation of tend-and-befriend in a non-human animal model.

Unfortunately, the way in which individuals develop a bias for looking at darker red faces remains largely unanswered. Despite the fact that females prefer to mate with males with darker faces (Dubuc et al. 2014a) and that previous research indicates that both adult males and females prefer to look at males of darker red faces, while juveniles do not have this preference (Dubuc et al. 2016), the studies presented here were unable to determine the exact age at which the bias develops. More precise work looking at the various characteristics of individuals (e.g., mating history) in the study, not merely their age, will possibly help clarify the differences in results between Dubuc et al. (2016) and the study presented here.

When advancing through juvenility to adolescence, many life history traits play a role. While dominance rank does indeed exert a major influence, this social trait may in fact just be moderating other factors. The most likely factors influenced by rank are access to resources which affects body condition. Body condition is known to exert strong influences on a variety of physiological processes, most importantly here, the hormonal cascade starting with the hypothalamic-pituitary-gonadal axis. Further studies must look at physiological body condition carefully across development and relate it to factors such as dominance rank, and perhaps other things such as maternal attachment or stress. The combination of social and physical factors would further complete the picture of the life history traits affecting sexual maturation.

Future studies with this population will expand on the proximate mechanisms and ultimate functions influencing life history traits. Previous research has shown that there are seasonal effects on the maturation of young females. Therefore, it is important to continue research in this population across multiple cohorts in order to disentangle which effects are consistent from one season to the next, and which may be specific to certain seasons.

APPENDIX 1

Ethogram

Continuous Focal sampling				
Code	**Behavior**	**Description**	**Modifier 1**	**Modifier 2**
“Bookend” behaviors (other behaviors can occur within these behaviors)				
c	Make Contact	Individual makes bodily contact with mom one for more than 5 seconds	Direction	M
d	Break Contact	Individual breaks contact with mom one for more than 5 seconds	Direction	M
p	Initiate proximity	Begins a bout of proximity within arms reach (without contact), either after breaking contact or after approaching	Direction	M
o	End proximity	Ends a proximity bout	Direction	M
State behaviors (will be analyzed as durations, percentages of time)				
f	Feed	Foraging, consuming, picking through, or preparing food	Food	Subject
r	Rest	Inactive, eyes open or closed		Subject
t	Travel	Travelling (walk, run, etc.)		Subject
Fo	Follow	Infant travels while following/being followed by mom (no distance criteria)	Direction	M
g	Groom	Subject engages with/engaged by another individual by picking through hair/skin (may or may not eat what is groomed)	Direction	Subject
Event behaviors (will be analyzed as frequencies)				
Juvenile Behaviors				
i	Self-directed behaviors	Actor does X to itself	Self	Subject
v	Vocalization	Infant vocalizes	Vocal	Subject
m	Tantrum	infant throws itself on ground screaming and jerking		Subject
q	Explore	Movement without clear purpose, exploration of area		Subject
Social Behaviors				
it	Infant Touch	Non-maternal individual briefly touches or holds the infant		Subject

k	Infant Kidnapping	Non-maternal individual prevents infant from going back to Mom for at least 60 sec		Subject
ab	Abuse	Subject is physically abused by mom	Abuse	M
gs	Groom solicit	Individual presents flank or rump for grooming, usually involves an exaggerated stretching motion	Direction	Subject
gd	Groom displacement	Individual interrupts grooming bouts between 2 others and replaces one of them	Direction	Subject
Ag	Contact aggression	Hit/bite/tackle (non-play)	Direction	subject
nc	Non-contact aggression	Lunging or chasing	Direction	Subject
ss	Submit Stay	Subject submits and remains in the same area	Direction	Subject
sl	Submit Leave	One individual moves to avoid another from it/displacement	Direction	Subject
fg	Grimace/Fear Grin	Fear grin or bared-teeth display	Direction	Subject
th	Threat	Stare and Open mouth threats	Direction	Subject
l	Lip-smacking	Lip-smacking.	Direction	Subject
mo	Mount	Mounting with no intromission	Direction	Subject
pr	Present	Presenting ano-genital area.	Direction	Subject
cop	Copulation	Mounting with intromission		Subject
cc	Ejaculation	Copulation with confirmed ejaculation		Subject
so	Solitary play	Infant plays with itself		Subject
sp	Social play	Infant plays with others	Direction	Subject
Observer codes				
un	Unknown	Observer cannot clearly see behavior		
pause	Pause	Observer needs to pause the observation		

REFERENCES

Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, *49*(3), 227-266.

Alberts, S. C., & Fitzpatrick, C. L. (2012). Paternal care and the evolution of exaggerated sexual swellings in primates. *Behavioral Ecology*, *23*(4), 699-706.

Anderson, C. M. (1986). Female age: Male preference and reproductive success in primates. *International Journal of Primatology*, *7*(3), 305-326.

Anderson, C. M., & Bielert, C. F. (1994). Adolescent exaggeration in female catarrhine primates. *Primates*, *35*(3), 283-300.

Aureli, F. (1997). Post-conflict anxiety in nonhuman primates: The mediating role of emotion in conflict resolution. *Aggressive Behavior*, *23*(5), 315-328.

Berard, J. (1999). A four-year study of the association between male dominance rank, residency status, and reproductive activity in rhesus macaques (Macaca mulatta). *Primates*, *40*(1), 159-175.

Bergman, T. J., Ho, L., & Beehner, J. C. (2009). Chest color and social status in male geladas (*Theropithecus gelada*). *International Journal of Primatology*, *30*(6), 791-806.

Berman, C. M. (1980). Early agonistic experience and rank acquisition among free-ranging infant rhesus monkeys. *International Journal of Primatology*, *1*(2), 153-170.

Bjorklund, D. F., Cormier, C., & Rosenberg, J. S. (2005). The evolution of theory of mind: Big brains, social complexity, and inhibition. *Young children's cognitive development: Interrelationships among executive functioning, working memory, verbal ability and theory of mind*, 147-174.

Blomquist, G. E. (2012). Female age of first reproduction at Cayo Santiago: heritability and shared environments. In *Bones, genetics, and behavior of rhesus macaques* (pp. 195-208). Springer, New York.

Borries, C., Lu, A., Ossi-Lupo, K., Larney, E., & Koenig, A. (2011). Primate life histories and dietary adaptations: a comparison of Asian colobines and macaques. *American journal of physical anthropology*, *144*(2), 286-299.

Brent, L. J. N., Semple, S., Dubuc, C., Heistermann, M., & MacLarnon, A. (2011). Social capital and physiological stress levels in free-ranging adult female rhesus macaques. *Physiology & behavior*, *102*(1), 76-83.

Bribiescas, R. G. (2001). Serum leptin levels and anthropometric correlates in Ache Amerindians of eastern Paraguay. *American journal of physical anthropology*, *115*(4), 297-303.

Bribiescas, R. G., & Anestis, S. F. (2010). Leptin associations with age, weight, and sex among chimpanzees (*Pan troglodytes*). *Journal of medical primatology*, *39*(5), 347-355.

Brown, S. L., Fredrickson, B. L., Wirth, M. M., Poulin, M. J., Meier, E. A., Heaphy, E. D., ... & Schultheiss, O. C. (2009). Social closeness increases salivary progesterone in humans. *Hormones and behavior*, *56*(1), 108-111.

Carlisle, K. S., Brenner, R. M., & Montagna, W. (1981). Hormonal regulation of sex skin in *Macaca nemestrina*. *Biology of Reproduction*, *25*(5), 1053-1063.

Carter, C. S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology*, *23*(8), 779-818.

Cavanaugh, J., Carp, S. B., Rock, C. M., & French, J. A. (2016). Oxytocin modulates behavioral and physiological responses to a stressor in marmoset monkeys. *Psychoneuroendocrinology*, *66*, 22-30.

Clutton-Brock, T. H. (1982). The functions of antlers. *Behaviour*, *79*(2), 108-124.

Coe, C. L., & Shirtcliff, E. A. (2004). Growth trajectory evident at birth affects age of first delivery in female monkeys. *Pediatric research*, *55*(6), 914-920.

Crockett, L. J. (1997). Cultural, historical, and subcultural contexts of adolescence: Implications for health and development. *Health risks and developmental transitions during adolescence*, 23-53.

Darwin, C. (1876). Sexual selection in relation to monkeys. *Nature*, *15*(366), 18-19.

Deardorff, J., Abrams, B., Ekwaru, J. P., & Rehkopf, D. H. (2014). Socioeconomic status and age at menarche: an examination of multiple indicators in an ethnically diverse cohort. *Annals of epidemiology*, *24*(10), 727-733.

Del Giudice, M., Ellis, B. J., & Shirtcliff, E. A. (2011). The adaptive calibration model of stress responsivity. *Neuroscience & Biobehavioral Reviews*, *35*(7), 1562-1592.

Depue, R. A., & Morrone-Strupinsky, J. V. (2005). A neurobehavioral model of affiliative bonding: Implications for conceptualizing a human trait of affiliation. *Behavioral and Brain Sciences*, *28*(3), 313-349.

Deschner, T., Heistermann, M., Hodges, K., & Boesch, C. (2004). Female sexual swelling size, timing of ovulation, and male behavior in wild West African chimpanzees. *Hormones and behavior*, *46*(2), 204-215.

Dixson, A. F. (1983). Observations on the evolution and behavioral significance of “sexual skin” in female primates. *Advances in the Study of Behavior*, *13*, 63-106.

Dixson, A. F. (1998). *Primate sexuality*. John Wiley & Sons, Ltd.

Dixson, A. F., & Anderson, M. J. (2004). Sexual behavior, reproductive physiology and sperm competition in male mammals. *Physiology & Behavior*, *83*(2), 361-371.

Domb, L. G., & Pagel, M. (2001). Sexual swellings advertise female quality in wild baboons. *Nature*, *410*(6825), 204-206.

Dominy, N. J., & Lucas, P. W. (2001). Ecological importance of trichromatic vision to primates. *Nature*, *410*(6826), 363-366.

dos Santos Silva, I., De Stavola, B. L., Mann, V., Kuh, D., Hardy, R., & Wadsworth, M. E. (2002). Prenatal factors, childhood growth trajectories and age at menarche. *International journal of epidemiology*, *31*(2), 405-412.

Dube, S. R., Anda, R. F., Felitti, V. J., Chapman, D. P., Williamson, D. F., & Giles, W. H. (2001). Childhood abuse, household dysfunction, and the risk of attempted suicide throughout the life span: findings from the Adverse Childhood Experiences Study. *Jama*, *286*(24), 3089-3096.

Dube, S. R., Miller, J. W., Brown, D. W., Giles, W. H., Felitti, V. J., Dong, M., & Anda, R. F. (2006). Adverse childhood experiences and the association with ever using alcohol and initiating alcohol use during adolescence. *Journal of Adolescent Health*, *38*(4), 444-e1.

Dubuc, C., Allen, W. L., Cascio, J., Lee, D. S., Maestripieri, D., Petersdorf, M., ... & Higham, J. P. (2016). Who cares? Experimental attention biases provide new insights into a mammalian sexual signal. *Behavioral Ecology*, arv117.

Dubuc, C., Brent, L. J., Accamando, A. K., Gerald, M. S., MacLarnon, A., Semple, S., ... & Engelhardt, A. (2009). Sexual skin color contains information about the timing of the fertile phase in free-ranging Macaca mulatta. *International Journal of Primatology*, *30*(6), 777-789.

Dubuc, C., Allen, W. L., Maestripieri, D., & Higham, J. P. (2014a). Is male rhesus macaque red color ornamentation attractive to females?. *Behavioral ecology and sociobiology*, *68*(7), 1215-1224.

Dubuc, C., Winters, S., Allen, W. L., Brent, L. J., Cascio, J., Maestripieri, D., ... & Higham, J. P. (2014b). Sexually selected skin colour is heritable and related to fecundity in a non-human primate. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*(1794), 20141602.

Ellis, B. J., & Garber, J. (2000). Psychosocial antecedents of variation in girls' pubertal timing: maternal depression, stepfather presence, and marital and family stress. *Child development*, *71*(2), 485-501.

Ellis, B. J. (2004). Timing of pubertal maturation in girls: an integrated life history approach. *Psychological bulletin*, *130*(6), 920.

Ellison, P. T. (2003). Energetics and reproductive effort. *American Journal of Human Biology*, *15*(3), 342-351.

Fairbanks, L. A. (1996). Individual differences in maternal style: causes and consequences for mothers and offspring. *Advances in the Study of Behavior, 25,* 579-611.

Haunhorst, C. B., Schülke, O., & Ostner, J. (2016). Opposite-sex social bonding in wild Assamese macaques. *American journal of primatology*, *78*(8), 872-882.

Gerald, M. S. (2001). Primate colour predicts social status and aggressive outcome. *Animal Behaviour*, *61*(3), 559-566.

Folstad, I., & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *The American Naturalist*, *139*(3), 603-622.

Foster, D. L., & Nagatani, S. (1999). Physiological perspectives on leptin as a regulator of reproduction: role in timing puberty. *Biology of Reproduction*, *60*(2), 205-215.

Girard-Buttoz, C., Higham, J. P., Heistermann, M., Wedegärtner, S., Maestripieri, D., & Engelhardt, A. (2011). Urinary C-peptide measurement as a marker of nutritional status in macaques. *PLoS One*, *6*(3), e18042.

Girolami, L., & Bielert, C. (1987). Female perineal swelling and its effects on male sexual arousal: an apparent sexual releaser in the chacma baboon (Papio ursinus). *International Journal of Primatology*, *8*(6), 651-661.

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biological psychiatry*, *49*(12), 1023-1039.

Heistermann, M., Palme, R., & Ganswindt, A. (2006). Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *American Journal of Primatology*, *68*(3), 257-273.

Higham, J. P., Heistermann, M., Saggau, C., Agil, M., Perwitasari-Farajallah, D., & Engelhardt, A. (2012). Sexual signalling in female crested macaques and the evolution of primate fertility signals. *BMC evolutionary biology*, *12*(1), 89.

Higham, J. P., Hughes, K. D., Brent, L. J., Dubuc, C., Engelhardt, A., Heistermann, M., ... & Stevens, M. (2011). Familiarity affects the assessment of female facial signals of fertility by free-ranging male rhesus macaques. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20110052.

Higham, J. P., MacLarnon, A. M., Heistermann, M., Ross, C., & Semple, S. (2009). Rates of self-directed behaviour and faecal glucocorticoid levels are not correlated in female wild olive baboons (Papio hamadryas anubis). *Stress*, *12*(6), 526-532.

Higham, J. P., Pfefferle, D., Heistermann, M., Maestripieri, D., & Stevens, M. (2013). Signaling in multiple modalities in male rhesus macaques: sex skin coloration and barks in relation to androgen levels, social status, and mating behavior. *Behavioral ecology and sociobiology*, *67*(9), 1457-1469.

Hodges, J. K., & Heistermann, M. (2011). Field endocrinology: monitoring hormonal changes in free-raning primates. In *Field and laboratory methods in primatology: A practical guide* (pp. 353-370) Cambridge University Press, Cambridge

Hoffman, C. L., Higham, J. P., Heistermann, M., Coe, C. L., Prendergast, B. J., & Maestripieri, D. (2011). Immune function and HPA axis activity in free-ranging rhesus macaques. *Physiology & behavior*, *104*(3), 507-514.

Holekamp, K. E., & Smale, L. (1991). Dominance acquisition during mammalian social development: the “inheritance” of maternal rank. *American Zoologist*, *31*(2), 306-317.

Hughes, K. D., Higham, J. P., Allen, W. L., Elliot, A. J., & Hayden, B. Y. (2015). Extraneous color affects female macaques’ gaze preference for photographs of male conspecifics. *Evolution and Human Behavior*, *36*(1), 25-31.

James-Todd, T., Tehranifar, P., Rich-Edwards, J., Titievsky, L., & Terry, M. B. (2010). The impact of socioeconomic status across early life on age at menarche among a racially diverse population of girls. *Annals of epidemiology*, *20*(11), 836-842.

Judge, P. G., & Mullen, S. H. (2005). Quadratic postconflict affiliation among bystanders in a hamadryas baboon group. *Animal Behaviour*, *69*(6), 1345-1355.

Kiess, W., Meidert, A., Dressendorfer, R. A., Schriever, K., Kessler, U., Konig, A., ... & Strasburger, C. J. (1995). Salivary cortisol levels throughout childhood and adolescence: relation with age, pubertal stage, and weight. *Pediatric Research*, *37*(4), 502-506.

Koski, S. E., de Vries, H., van de Kraats, A., & Sterck, E. H. (2012). Stability and change of social relationship quality in captive chimpanzees (*Pan troglodytes*). *International Journal of Primatology*, *33*(4), 905-921.

Landis, J. R., & Koch, G. G. (1977). An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics*, 363-374.

Leenstra, T., Petersen, L. T., Kariuki, S. K., Oloo, A. J., Kager, P. A., & Ter Kuile, F. O. (2005). Prevalence and severity of malnutrition and age at menarche; cross-sectional studies in adolescent schoolgirls in western Kenya. *European journal of clinical nutrition*, *59*(1), 41-48.

Leigh, S. R., & Blomquist, G. E. (2011). Life History. In *Primates in Perspective,* Oxford University Press

Lyons, D. M., & Parker, K. J. (2007). Stress inoculation-induced indications of resilience in monkeys. *Journal of traumatic stress*, *20*(4), 423-433.

Machatschke, I. H., Dittami, J., & Wallner, B. (2006). Morphometric and hormonal changes during the chimpanzee menstrual cycle. *Journal of medical primatology, 35*(6), 331-340.

Maestripieri, D. (2002). Parent–offspring conflict in primates. *International Journal of Primatology*, *23*(4), 923-951.

Maestripieri, D. (2005). Effects of early experience on female behavioural and reproductive development in rhesus macaques. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1569), 1243-1248.

Maestripieri, D., Higley, J. D., Lindell, S. G., Newman, T. K., McCormack, K. M., & Sanchez, M. M. (2006). Early maternal rejection affects the development of monoaminergic systems and adult abusive parenting in rhesus macaques (Macaca mulatta). *Behavioral neuroscience*, *120*(5), 1017.

Maestripieri, D., Lindell, S. G., & Higley, J. D. (2007). Intergenerational transmission of maternal behavior in rhesus macaques and its underlying mechanisms. *Developmental psychobiology*, *49*(2), 165-171.

Mandalaywala, T. M., Higham, J. P., Heistermann, M., Parker, K. J., & Maestripieri, D. (2014). Physiological and behavioural responses to weaning conflict in free-ranging primate infants. *Animal behaviour*, *97*, 241-247.

Maner, J. K., & Miller, S. L. (2014). Hormones and social monitoring: Menstrual cycle shifts in progesterone underlie women's sensitivity to social information. *Evolution and Human Behavior*, *35*(1), 9-16.

Maner, J. K., Miller, S. L., Schmidt, N. B., & Eckel, L. A. (2010). The endocrinology of exclusion rejection elicits motivationally tuned changes in progesterone. *Psychological Science*.

Manson, J. H. (1992). Measuring female mate choice in Cayo Santiago rhesus macaques. *Animal Behaviour*, *44*, 405-416.

Marty, J. S., Higham, J. P., Gadsby, E. L., & Ross, C. (2009). Dominance, coloration, and social and sexual behavior in male drills Mandrillus leucophaeus. *International Journal of Primatology*, *30*(6), 807-823.

McClintock, M. K., & Herdt, G. (1996). Rethinking puberty: The development of sexual attraction. *Current Directions in Psychological Science*, *5*(6), 178-183.

Nowak-Szczepanska, N., Gomula, A., Ipsen, M. J., & Koziel, S. (2016). Different effects of living conditions on the variation in BMI and height in children before the onset of puberty. *European journal of clinical nutrition*, *70*(6), 662-666.

Nunn, C. L. (1999). The evolution of exaggerated sexual swellings in primates and the graded-signal hypothesis. *Animal Behaviour*, *58*(2), 229-246.

Oliver, L. N., & Hayes, M. V. (2005). Neighbourhood socio-economic status and the prevalence of overweight Canadian children and youth. *Canadian Journal of Public Health/Revue Canadienne de Sante'e Publique*, 415-420.

Ozaza, H., & Gould, K. G. (1982). Demonstration and characterization of estrogen receptor in chimpanzee sex skin: correlation between nuclear receptor levels and degree of swelling. *Endocrinology*, *111*(1), 125-131.

Parker, K. J., & Maestripieri, D. (2011). Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. *Neuroscience & Biobehavioral Reviews*, *35*(7), 1466-1483.

Petrullo, L. A., Mandalaywala, T. M., Parker, K. J., Maestripieri, D., & Higham, J. P. (2016). Effects of early life adversity on cortisol/salivary alpha-amylase symmetry in free-ranging juvenile rhesus macaques. *Hormones and behavior*, *86*, 78-84.

Phillips, K. A., Bales, K. L., Capitanio, J. P., Conley, A., Czoty, P. W., t Hart, B. A., ... & Nathanielsz, P. W. (2014). Why primate models matter. *American journal of primatology*, *76*(9), 801-827.

Plavcan, J. M., van Schaik, C. P., & Kappeler, P. M. (1995). Competition, coalitions and canine size in primates. *Journal of human evolution*, *28*(3), 245-276.

Price, T. (1998). Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *353*(1366), 251-260.

Rahkovskaya, M., & Heistermann, M. Faecal glucocorticoid metabolite (fGCM) concentrations of rhesus macaques on Cayo Santiago, Puerto Rico. Unpublished raw data n.s.

Rawlins, R. G., & Kessler, M. J. (1986). *The Cayo Santiago macaques: History, behavior, and biology*. SUNY Press.

Resko, J. A., Goy, R. W., Robinson, J. A., & Norman, R. L. (1982). The pubescent rhesus monkey: some characteristics of the menstrual cycle. *Biology of reproduction*, *27*(2), 354-361.

Rhodes, L., Argersinger, M. E., Gantert, L. T., Friscino, B. H., Hom, G., Pikounis, B., ... & Rhodes, W. L. (1997). Effects of administration of testosterone, dihydrotestosterone, oestrogen and fadrozole, an aromatase inhibitor, on sex skin colour in intact male rhesus macaques. *Journal of Reproduction and Fertility*, *111*(1), 51-57.

Rigaill, L., Higham, J. P., Lee, P. C., Blin, A., & Garcia, C. (2013). Multimodal sexual signaling and mating behavior in olive baboons (Papio anubis). *American journal of primatology*, *75*(7), 774-787.

Rodrigues, M. A. (2013). *Stress and sociality in a patrilocal primate: Do female spider monkeys tend-and-befriend?* (Doctoral dissertation, The Ohio State University).

Sanchez, M. M., Mccormack, K., Grand, A. P., Fulks, R., Graff, A., & Maestripieri, D. (2010). Effects of sex and early maternal abuse on adrenocorticotropin hormone and cortisol responses to the corticotropin-releasing hormone challenge during the first 3 years of life in group-living rhesus monkeys. *Development and psychopathology*, *22*(01), 45-53.

Schultheiss, O. C., Wirth, M. M., & Stanton, S. J. (2004). Effects of affiliation and power motivation arousal on salivary progesterone and testosterone. *Hormones and behavior*, *46*(5), 592-599.

Setchell, J. M., & Jean Wickings, E. (2005). Dominance, status signals and coloration in male mandrills (Mandrillus sphinx). *Ethology*, *111*(1), 25-50.

Shirtcliff, E. A., Allison, A. L., Armstrong, J. M., Slattery, M. J., Kalin, N. H., & Essex, M. J. (2012). Longitudinal stability and developmental properties of salivary cortisol levels and circadian rhythms from childhood to adolescence. *Developmental psychobiology*, *54*(5), 493-502.

Silk, J. B., Beehner, J. C., Bergman, T. J., Crockford, C., Engh, A. L., Moscovice, L. R., ... & Cheney, D. L. (2010a). Female chacma baboons form strong, equitable, and enduring social bonds. *Behavioral Ecology and Sociobiology*, *64*(11), 1733-1747.

Silk, J. B., Beehner, J. C., Bergman, T. J., Crockford, C., Engh, A. L., Moscovice, L. R., ... & Cheney, D. L. (2010b). Strong and consistent social bonds enhance the longevity of female baboons. *Current Biology*, *20*(15), 1359-1361.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews*, *24*(4), 417-463.

Stearns, S. C. (1976). Life-history tactics: a review of the ideas. *The Quarterly review of biology*, *51*(1), 3-47.

Stearns, S. C. (1992). *The evolution of life histories* (Vol. 249). Oxford: Oxford University Press.

Stevens, M., Stoddard, M. C., & Higham, J. P. (2009). Studying primate color: towards visual system-dependent methods. *International Journal of Primatology*, *30*(6), 893-917.

Surridge, A. K., Osorio, D., & Mundy, N. I. (2003). Evolution and selection of trichromatic vision in primates. *Trends in Ecology & Evolution*, *18*(4), 198-205.

Taylor, S. E. (2006). Tend and befriend biobehavioral bases of affiliation under stress. *Current directions in psychological science*, *15*(6), 273-277.

Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A., & Updegraff, J. A. (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychological review*, *107*(3), 411.

Taylor, S. E., & Master, S. L. (2011). Social responses to stress: The tend-and-befriend model. *The handbook of stress science: Biology, psychology, and health*, 101-109.

Trickett, P. K., & Putnam, F. W. (1993). Impact of child sexual abuse on females: Toward a developmental, psychobiological integration. *Psychological Science*, *4*(2), 81-87.

Turner, H. A., Vanderminden, J., Finkelhor, D., Hamby, S., & Shattuck, A. (2011). Disability and victimization in a national sample of children and youth. *Child maltreatment*, *16*(4), 275-286.

Waitt, C., Gerald, M. S., Little, A. C., & Kraiselburd, E. (2006). Selective attention toward female secondary sexual color in male rhesus macaques. *American Journal of Primatology*, *68*(7), 738-744.

Walters, J. R. (1987). Transition to adulthood. In *Primate Societies* (pp. 358-369), The University of Chicago Press, Chicago

Wichmann, M. W., Zellweger, R., DeMaso, C. M., Ayala, A., & Chaudry, I. H. (1996). Mechanism of immunosuppression in males following trauma-hemorrhage: critical role of testosterone. *Archives of Surgery*, *131*(11), 1186-1192.

Wilson, M. E., Gordon, T. P., Blank, M. S., & Collins, D. C. (1984). Timing of sexual maturity in female rhesus monkeys (Macaca mulatta) housed outdoors. *Journal of reproduction and fertility*, *70*(2), 625-633.

Wilson, M. E., Walker, M. L., & Gordon, T. P. (1983). Consequences of first pregnancy in rhesus monkeys. *American journal of physical anthropology*, *61*(1), 103-110.

Young, C., Majolo, B., Schülke, O., & Ostner, J. (2014). Male social bonds and rank predict supporter selection in cooperative aggression in wild Barbary macaques. *Animal Behaviour*, *95*, 23-32.

Zahavi, A. (1975). Mate selection—a selection for a handicap. *Journal of theoretical Biology*, *53*(1), 205-214.

Zehr, J. L., Van Meter, P. E., & Wallen, K. (2005). Factors regulating the timing of puberty onset in female rhesus monkeys (Macaca mulatta): role of prenatal androgens, social rank, and adolescent body weight. *Biology of reproduction*, *72*(5), 1087-1094.

Zinner, D., Alberts, S. C., Nunn, C. L., & Altmann, J. (2002). Evolutionary biology (Communication arising): Significance of primate sexual swellings. *Nature*, *420*(6912), 142-143.