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## Social and emotional predictors of the tempo of puberty in female rhesus monkeys

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### Abstract

A cascade of neuroendocrine events regulates the initiation and progression of female puberty. However, the factors that determine the timing of these events across individuals are still uncertain. While the consequences of puberty on subsequent emotional development and adult behavior have received significant attention, what is less understood are the social and environmental factors that actually alter the initiation and progression of puberty. In order to more fully understand what factors influence pubertal timing in females, the present study quantified social and emotional behavior; stress physiology; and growth and activity measures in juvenile female rhesus monkeys to determine what best predicts eventual puberty. Based on previous reports, we hypothesized that increased agonistic behavior resulting from subordinate status in their natal group, in combination with slowed growth, reduced prosocial behavior, and increased emotional reactivity would predict delayed puberty. The analyses were restricted to behavioral and physiological measures obtained prior to the onset of puberty, defined as menarche. Together, our findings indicate that higher rates of aggression but lower rates of submission received from group mates; slower weight gain; and greater emotional reactivity, evidenced by higher anxiety, distress and appeasing behaviors, and lower cortisol responsivity in response to a potentially threatening situation, predicts delayed puberty. Together the combination of these variables accounted for 58% of the variance in the age of menarche, 71% in age at first ovulation, and 45% in the duration of adolescent sterility. While early puberty may be more advantageous for the individual from a fertility standpoint, it presents significant health risks, including increased risk for a number of estrogen dependent cancers and as well as the emergence of mood disorders during adulthood. On the other hand, it is possible that increased emotional reactivity associated with delayed puberty

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Mark Wilson: PI of grant; finalized data analysis; lead author

Shannon Bounar: technician who helped collect and analyze data

Jodi Godfrey: technician who helped collect and analyze data

Vasiliki Michopoulos: graduate student who assisted in summarizing group social behavioral data

Melinda Higgins: statistician

Mar Sanchez: co-investigator on grant; supervised emotionality tests; contributed significantly to writing the manuscript

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could persist, increasing the risk for emotional dysregulation to socially challenging situations. The data argue for prospective studies that will determine how emotional reactivity shown to be important for pubertal timing is affected by early social experience and temperament, and how these stress-related variables contribute to body weight accumulation, affecting the neuroendocrine regulation of puberty.

## Keywords

menarche; first ovulation; puberty; emotionality; and social stress

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## Introduction

The initiation and progression of female puberty is regulated by a cascade of neuroendocrine events leading to menarche and eventual first ovulation (Terasawa and Fernandez, 2001). However, the factors that determine the timing of these events across individuals are still uncertain. In addition to the importance of gene polymorphisms (Elks and Ong, 2011; Towne et al., 2005), a number of social and environmental signals likely alter the timing of puberty (Buck Louis et al., 2008). Apart from its importance for fertility, alterations in pubertal timing have lasting effects on both physical and emotional health (Golub et al., 2008; Walvoord, 2010). Because pubertal increases in gonadal steroids further stimulate brain maturation and behavioral development (Ahmed et al., 2008; Schulz et al., 2009; Sisk and Zehr, 2005; Vigil et al., 2011), it is possible that the hormonal consequences of puberty might prepare adolescents to adapt successfully to their social environment regardless of pubertal timing. However, this is not necessarily the case, as a number of studies show that early puberty is often associated with increased risk for mood and anxiety disorders as well as social impairments during late adolescence and adulthood, particularly in girls (Graber et al., 1997; Mendle et al., 2007; Nelson et al., 2005; Reardon et al., 2009; Zehr et al., 2007). The mechanisms responsible for the emergence of socio-emotional problems in early developing individuals are poorly understood, but likely reflect the interaction of the social demands of adolescence and a developing brain (Angold and Costello, 2006; Blumenthal et al., 2009; Casey et al., 2010; Patton and Viner, 2007; Walker et al., 2004). While the consequences of puberty on subsequent emotional development and adult behavior have received significant attention, what is less understood are the social, physiological, and environmental factors that actually alter the initiation and progression of puberty.

Because exposure to psychosocial stressors has an inhibitory effect on the neuroendocrine regulation of fertility, particularly in women (Berga and Loucks, 2005; Warren and Fried, 2001) as well as nonhuman females (Baker et al., 2006; Bethea et al., 2008; Kaplan et al., 2010b; Pope et al., 1986; Wagenmaker et al., 2008; Xiao et al., 2002), one critical factor affecting puberty timing could be exposure to psychosocial stress and activation of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis prior to puberty, during childhood and adolescence. Indeed, social subordination in female macaques, known to be a potent psychosocial stressor in adults (Jarrell et al., 2008a; Michopoulos et al., 2012a; Michopoulos et al., 2012b; Shively et al., 1997b), delays puberty and the occurrence of first ovulation (Wilson et al., 1986a; Zehr et al., 2005). Furthermore, this delay in puberty is better predicted by social subordination during adolescence rather than at birth (Schwartz et al., 1985), suggesting that social stressor exposure imposed by subordination during development is a critical factor. Experimental studies in female rodents provide further evidence that an increase in stress hormones postnatally delays sexual maturation (Alves et al., 1993).

However, a different, more complex pattern emerges in studies of children (Mishra et al., 2009). Whereas positive family relations and the presence of an adult male engaged in child rearing predict a later pubertal age, exposure to a disruptive family environment is often associated with accelerated, not delayed, puberty (Ellis and Essex, 2007; Ellis and Garber, 2000; Ellis et al., 2011; Hulanicka et al., 2001; Kim and Smith, 1998). More recent studies show that the effects of an absent father on accelerating indices of puberty may be restricted to families of high socioeconomic status (Deardorff et al., 2011). In addition, the data suggest reactivity to an acute stressor interacts with the family environment to predict puberty timing (Ellis et al., 2011). The data are explained by a life-history theory that argues girls living in stressful environments have accelerated sexual maturation to initiate fertility and increase chances of producing more offspring (Ellis et al., 2011). However, the proximate neuroendocrine mechanisms produced by the stressful experiences during adolescence to account for the accelerated initiation of puberty are unknown. As noted previously (Cameron, 2004), it is difficult to reconcile how a stressful home environment could accelerate puberty, given that chronic stressor exposure consistently results in reproductive compromise in adults (Berga and Loucks, 2005; Warren and Fried, 2001). This is underscored by data showing girls with high urinary cortisol levels have a delayed growth spurt and a later age at menarche (Shi et al., 2011), and the data showing that adverse physical and psychological effects associated with wartime also delays age of menarche (Tahirovic, 1998).

Clearly, social, experiential, and innate temperamental factors can mitigate or accentuate the consequences of stress on puberty, as individuals can develop a differential neurobehavioral response that is shaped by a nurturing and supportive social environment in the face of stressor exposure during adolescence (Koolhaas et al., 1999). Importantly, this differential sensitivity-reactivity may be more predictive of pubertal timing than exposure to adverse experience during adolescence alone (Boyce and Ellis, 2005; Ellis et al., 2011). Thus, the degree of emotional and social reactivity (Barker et al., 2010; Freyberg, 2009; Heim and Nemeroff, 2001; McLaughlin et al., 2010; Richards et al., 1998; Slutske et al., 2010; Spear, 2000) could be predictive of pubertal timing. However, few studies have examined whether prepubertal emotional reactivity, as a proxy for the behavioral response to a socially stressful environment, affect the timing of puberty (Reardon et al., 2009). As noted above, measures of increased anxiety behaviors and reports of a more stressful family environment are often correlated with an eventual earlier age at menarche (Graber et al., 1995). In contrast, other studies report little or no predictive relation between anxiety and the timing of menarche (Kim and Smith, 1998; Meschke et al., 2003).

An unequivocal predictor of puberty in children (Tanner and Whitehouse, 1975) and monkeys (Tanner and Wilson, 1990) is the timing of the adolescent growth spurt. It is, therefore, not surprising that postnatal growth positively predicts pubertal timing in girls (Davison et al., 2003) and female monkeys (Zehr et al., 2005) and is consistent with emerging evidence that excess body fat in childhood results in early puberty in girls (Burt Solorzano and McCartney, 2010; Freedman et al., 2003; Reardon et al., 2009). While BMI was a significant predictor of early puberty in girls, it was unrelated to the effect of the absence of a father in girls (Deardorff et al., 2011), suggesting both may be independent predictors of puberty. On the other hand, although statural growth during prepuberty is slowed in children living in a disruptive home environment (Montgomery et al., 1997), stress reactivity in children is predictive of increased adiposity in boys and girls (Roemmich et al., 2007), perhaps the result of emotional feeding of high caloric foods in response to the stressful environment (Epel et al., 2004).

In order to more fully understand what factors influence pubertal timing in females, the present study quantified social and emotional behavior, stress physiology, and growth and

activity measures in juvenile female rhesus monkeys to determine what best predicts eventual puberty. We hypothesized that that increased submissive behavior in response to aggression by group mates resulting from subordinate social status, in combination with slowed growth, reduced prosocial behavior, and increased emotional reactivity would predict delayed puberty. This prediction is based on previous studies in monkeys showing social subordination delays puberty (Schwartz et al., 1985; Wilson et al., 1986a; Zehr et al., 2005) and contrasts most (Deardorff et al., 2011; Ellis and Essex, 2007; Ellis and Garber, 2000; Ellis et al., 2011; Hulanicka et al., 2001; Kim and Smith, 1998) but not all (Shi et al., 2011; Tahirovic, 1998) data derived from girls living in stressful environments. Furthermore, because puberty is not a unitary event, but rather reflects a continuum from onset, progression, and culminating eventually in first ovulation (Buck Louis et al., 2008), the study independently evaluated what factors influenced each stage of development.

## Methods

Subjects were thirty-eight female juvenile rhesus monkeys (*Macaca mulatta*) born at the Yerkes National Primate Research Center Field Station and were raised as members of one of four large social groups of 60–80 monkeys in outdoor, half-acre compounds with an attached indoor area. Group composition included 7 to 10 matriline with multiple related adult females and their juvenile offspring as well as two to four adult males. At the beginning of the study, subjects ranged from 10 to 16 mo of age, some 8 to 10 months prior to expected age at menarche in rhesus monkeys in this colony (Wilson et al., 1988a). Animals were fed standard, low fat high fiber monkey chow (Purina Mills International, Lab Diets, #5038, St. Louis MO) twice-daily, ad libitum, with a daily supplementation of seasonal fresh fruit and vegetables and had continuous access to water. The Emory University Institutional Animal Care and Use Committee approved all procedures in accordance with the Guide for Use of Laboratory Animals and the Animal Welfare Act. A linear dominance hierarchy defines the social organization of a rhesus monkey social group, regardless of size, with matriline or families ranking over one another (Bernstein, 1970). Because macaques have a matrilineal social organization, offspring assume the relative rank of their mothers. Thus, the rank of a female rhesus monkey matches that of her mother's and others in the matriline during development and into adulthood (Bernstein, 1970). As a result of their low social status, lower-ranking animals have less control over their social and physical environment (Sapolsky, 2005). In adult female macaques, subordinate status is associated with dysregulation of the LHPA axis, most consistently shown by diminished glucocorticoid negative feedback (Jarrell et al., 2008b; Shively, 1998; Wilson et al., 2008). Social subordination in adult female macaques is a well-established model to study the adverse health effects of psychosocial stress on a number of phenotypes ranging from reproductive compromise (Kaplan et al., 2010a; Michopoulos et al., 2009), risk for cardiovascular disease (Kaplan et al., 2009), emotional dysregulation (Shively et al., 2006; Shively et al., 1997a), immune function (Paiardini et al., 2009), addiction (Morgan et al., 2002), and emotional feeding (Arce et al., 2010; Michopoulos et al., 2012c). For the present study, the relative dominance rank of each subject within her natal group was determined throughout the study period based on the outcome of dyadic agonistic interactions. A subordinate female is one who unequivocally emits a submissive behavior to all animals ranking above her (Bernstein, 1976). Animals were assigned a relative rank within her group that was calculated as the ratio of her rank to the total number of animals in the group, exclusive of animals less than 12 months of age. Thus, a subject ranked 25 out of a group of 100 animals would receive a relative rank of 0.25. These determinations of rank were confirmed during the social group observations (see below) as well as during daily rounds made by research staff throughout the study period.

## Assessment of Puberty

Puberty is not a discrete reproductive event but represents a continuum that eventually leads to fertility. Female macaques, like humans, typically have first ovulation long before adult stature and size are attained (Wilson and Tanner, 1991), such that the interval between puberty onset and completion (first ovulation) is termed adolescent sterility (Hartman, 1952). Thus, we divided this continuum into three phases: puberty onset, adolescent sterility and completion or first ovulation. The initiation of puberty was operationally defined as the appearance of perineal swelling or menarche based on 4 to 5 observations each week (Wilson et al., 1986a; Wilson et al., 1988b). The degree of perineal coloration and swelling was each rated on a 3-point scale with 0.5 increments. Prior to puberty onset, a serum sample was obtained biweekly from each subject. Once menarche or perineal changes were first observed, a serum sample was collected twice weekly for the determination of serum progesterone to identify first ovulation from the luteal phase increase in serum progesterone (Wilson et al., 1986a; Wilson et al., 1988b). This protocol was maintained until first ovulation was determined for each subject. The period of adolescent sterility was defined as the number of months between menarche and first ovulation. Because rhesus monkeys housed outdoors have ovulations restricted to the Fall and Winter months from Sept - March (Walker et al., 1984), first ovulation but not menarche is also restricted to this period (Wilson et al., 1988b), regardless of season of birth (Wilson and Gordon, 1989). Subjects were born during the same birth season in 2007, ranging from March through Oct, with a median of June 3. Ninety four percent of the subjects were born from March – July, with one each in August and October. Thus, first ovulations occur during the breeding season at ~ 30 mo (“early”) or ~42 mo (“late”).

All subjects had been habituated to the access and conscious venipuncture procedures at the start of the study using previously described methods (Wilson et al., 1986a; Wilson et al., 1988b) that does not disturb adult fertility (Walker et al., 1982) or development (Wilson et al., 1986a; Wilson et al., 1988b). Subjects were trained to individually move from their outdoor enclosure into the caged area of the attached indoor quarters. From there they moved to a small transfer container so they could be placed in a cage or relocated to the behavioral testing facility (see below). Females were trained to voluntarily place their leg through a small opening in the front of the holding cage so that a blood sample could be obtained from the saphenous vein without the use of anesthesia.

## Social Behavior

Behavioral observations were collected monthly from the average age of  $14.3 \pm 0.24$  until  $26.0 \pm 0.24$  mo. This interval encompasses the transition from the prepubertal period up to the expected earliest age of menarche. At each sampling interval a 30-minute focal observation (Altmann and Altmann, 1977) was done for each subject in which all behaviors were recorded using an established ethogram (Maestriperi et al., 2006). Behavioral categories observed included affiliative (proximity, grooming, contact), agonistic (aggression and submission), anxiety-like (scratch, yaw, body shake) (Schino et al., 1996; Troisi et al., 1991), playful and sexual behaviors. Data were recorded using an Acer Netbook using a program that captures initiator, the behavior, the recipient, and a time stamp program (Graves and Wallen, 2006). Inter-observer reliability exceeded 92%.

## Emotional Behavior

Subjects received two standardized tests of emotionality at an average age of  $18.37 \pm 0.11$  mo, some 8 months prior to the earliest expected age of menarche. The approach – avoidance (AA) and human intruder (HI) tasks are ethologically relevant laboratory tests that evoke very strong and distinct behavioral responses to novel stimuli of varying threatening intensities in rhesus monkeys, including defensive responses reflecting

underlying states of anxiety and fear, as well as aggressive and submissive responses, and exploration (Kalin and Shelton, 1989; Machado et al., 2009; Meunier et al., 1999). These are unconditioned responses present very early in life and stable throughout development (Kalin et al., 1991) and have been used to quantify differences in fearful behaviors in infant and juvenile rhesus monkeys (Bethea et al., 2004; Grand et al., 2005). To conduct these emotionality tests, animals were accessed as described above and transported to a cage in a behavioral testing room located in a facility some two minutes from each compound. Prior to the initiation of the emotionality tests, animals were habituated to the laboratory environment and procedures by being removed from their social group and placed in the testing cage for 30 minutes before returning to their group. This brief social separation is a potent stressor in monkeys and activates the LHPA axis (Arce et al., 2010). Thus, at the first 30-minute habituation period, a baseline serum sample was collected at the time of access from her group and again at the end of the 30-minute habituation period for stress-induced cortisol secretion determination. Two additional 15-minute habituations to the test room and cage were done within a week of the first habituation and prior to the start of testing.

Test cages measured 0.7 by 0.6 by 0.8 m (L x W x H) and were placed on top of a stand. One side was made of transparent Plexiglas to enable view of the human during the HI paradigm (and videotaping) and the other side of the cage had vertical bars with a removable tray measuring 0.7 by 0.4 by 0.3 m for placement of objects in the AA test. The tray had a solid metal bottom and Plexiglas sides so that the videocamera could capture object manipulation during the AA task. A Sony digital video camera (model DCR-SR85) was placed in front of each cage to record behavior for later scoring and a white noise generator was used to minimize interference from extraneous sounds outside of the testing room. A curtain separated the cage from a door where the experimenter entered the room.

The AA task was presented the week following the last habituation and consisted of six five-minute sessions where a different novel object was placed in the Plexiglas tray for each session (Meunier et al., 1999). A solid metal sheet was placed in between the monkey and the tray after each session so that the animal would not see the experimenter replacing the objects. Three novel objects were selected to provoke a fearful response and three objects were chosen as neutral. A positive food reward (jellybean) was placed adjacent to each object in each session. Subjects were able to reach through the bars in the testing cage to manipulate and bite items in the box, but items were not small enough to fit through the hole (to avoid retrieval). The 6 objects were presented in the same order across subjects and were chosen based on characteristics previously shown to evoke fearful or defensive responses in this species (Bethea et al., 2004; Dettling et al., 2002b; Meunier et al., 1999; Williamson et al., 2003). For the present analysis, we only report responses to a battery operated toy pig that moved and emitted sounds (fear evoking).

A week following AA task completion, the HI task (Kalin and Shelton, 1989) was presented. The HI paradigm is comprised of three consecutive ten-minute conditions, including an alone (alone), intruder profile (profile) and intruder stare condition (stare). During the first condition, the subject was alone in the testing room and cage. This condition elicits distress responses (vocalizations, locomotion) and exploration. At the end of the 10 min alone condition, an experimenter unfamiliar to the subject entered the testing room and sat with his/her facial profile towards the monkey. During the profile condition rhesus monkeys typically stop vocalizing and become behaviorally inhibited (freeze) while scanning the environment/intruder. For the third condition, the experimenter turned to face the animal and made continuous eye contact for the entire ten minutes (stare). Direct eye contact is a threatening behavior for rhesus macaques, which typically stop freezing and display aggressive or submissive behaviors towards the intruder.

The videos were scored using an ethogram (Machado and Bachevalier, 2006) established to quantify individual behaviors. Inter-observer reliability was greater than 92%. Because different behaviors may reflect similar emotional reactions to stimuli, a Principal Components Analysis (PCA) using a rotation method of Oblimin with Kaiser Normalization was performed using SPSS 19.0 software for both the AA and HI data together to consolidate behaviors into similar patterns of responses that represent the most prominent behavioral phenotypes emerging during these tests of emotionality (see Table 1). Similar PCA analyses have been previously performed to examine the interconnections of behavioral responses during the HI and other emotionality tests in rhesus monkeys (Williamson et al., 2003). Behaviors that had a loading score of 0.45 or less were excluded.

Eight behaviors from the AA and eight from the HI were included in the analysis based on their relevance to rhesus monkey emotional reactivity during the tasks (i.e., fear, anxiety, distress, exploration, aggression, submission), as detailed above. All but two behaviors contributed uniquely to one of the components. The first component was labeled “Distress” based on the positive loading of the coo vocalization during both the HI and AA, consistent with previous reports of PCA loadings for rhesus monkeys (Williamson et al., 2003). This behavior is thought to reflect distress during threatening situations, as described above for the HI (Kalin and Shelton, 1998). In addition, withdrawal from the stimulus and locomotion in the cage during both the AA also loaded positively. In this context, these behaviors may function to move away from and limit interaction with the stimulus. Similarly, inspect object during the AA loaded negatively, indicating that animals that showed a high frequency of coo vocalizations and withdrawing had reduced frequency of visually exploring the object. Finally, the duration of freezing during the profile condition of the HI, reflecting fearfulness (Kalin and Shelton, 1998), also loaded negatively. This component may, thus, reflect two response strategies: an active coping style where animals coo call for help and locomote to remove herself from the potentially threatening stimulus or a passive style in which the animal freezes and visually inspects it.

The second component that emerged included lipsmack and avert gaze – look away from the intruder in the HI both of which loaded positively. Lip smack is described as a “pacificatory behavior” (Altmann, 1962) that functions to neutralize agonistic behavior from another animal, in this context the intruder. Others have considered lipsmack as an affiliative behavior (Machado and Bachevalier, 2008) that may accurately reflect an animal’s attempt to neutralize a potentially threatening interaction. Breaking eye contact by averting gaze or looking away from the intruder may function to achieve the same goal. Given this rationale, this component was labeled “Appeasement”.

All behaviors in the third component loaded positively and included self scratch – yawn in the HI, both established measures of anxiety in monkeys (Schino et al., 1996). Also loading were withdraw - locomotion during the intruder condition of the HI test as well as breaking eye contact (avert gaze – look away), behaviors that may function to avoid interaction with the object. Finally, threaten the object in the AA also loaded positively. While threat is considered an aggressive behavior for this species (Altmann, 1962), in the context of the AA and the other behaviors within this component it may more reflect non-contact, defensive aggression, as previously reported in other studies (Meunier et al., 1999). Taken together, this component was labeled “Anxiety”.

The fourth component was labeled Defensive aggression. Tooth grind from the HI loaded positively and is considered an anxiety or distress behavior in social contexts (Machado and Bachevalier, 2006) and during the HI (Williamson et al., 2003). Also loading positively were bite object and explore cage during the AA, with the former clearly an aggressive

behavior and the later potentially reflecting a displacement behavior in response to the object, based on the context of the other behaviors within this component.

The fifth component was comprised of three behaviors and was labeled “Vigilance”. Inspect object during the AA loaded positively while threat towards the intruder during the HI and freeze duration during the profile condition of the HI loaded negatively. The rationale for labeling this component vigilance was that visual inspection of the stimulus during the AA loaded with low fear and aggression towards the HI.

### Growth and activity

Body weights were collected bi-weekly from each subject. Weight gain was determined from the increase in weight from the start of the study to 26 months of age. Actical accelerometers (MiniMitter), attached to collars (Primate Products) were used to provide an unbiased measure of physical activity (Hunnell et al., 2007; Sullivan et al., 2006). Every subject wore Acticals for 6 days at a mean age of  $16.2 \pm 0.2$  mo. Activity was sampled in 30 second epochs. For the present analysis, average daytime (0700 to 1900 hr) activity across the 6 days was calculated for each subject.

### Hormone assays

Assays were performed in the Yerkes Biomarkers Core Lab. Serum cortisol was determined using a commercially available radioimmunoassay kit (Beckman-Coulter/DSL, Webster TX). Using 25  $\mu$ l, the assay has a range from 0.50 to 60  $\mu$ g/dl with an intra- and inter-assay CV of 4.9% and 8.7%, respectively. Serum progesterone was quantified by a previously validated radioimmunoassay (Pazol et al., 2004) that has a sensitivity of 0.2 ng/ml (assaying 100  $\mu$ l of serum) and an inter- and intra-assay coefficient of variation of 8.7% and 5.1%, respectively.

### Statistical analysis

The intent of this study was to identify social, physiological and emotionality factors that significantly accounted for variance in three parameters of puberty: age at menarche, duration of adolescent sterility, and age at first ovulation. All data were derived from the juvenile phase of development up to the expected earliest age of menarche (25 mo) because including data following the initiation of puberty would make it difficult to disentangle predictors from consequences of reproductive maturation. Data were summarized as mean  $\pm$  standard deviation (sd) for descriptive purposes. A list of the variables that contributed to the analysis are shown in Table 1. Simple bivariate (Pearson product moment) correlations were run to describe the relation between the phenotypes and, in some cases, parameters of puberty. Multivariate regression models were used with stepwise variable selection methods to identify factors that uniquely and significantly explained variance in the outcome measure. For the data derived from the human intruder and approach – avoidance tests, the component scores, representing the emotional phenotypes, were used in the regression analyses rather than the rates or durations of individual behaviors. Statistical values of  $p < 0.05$  were considered significant.

## Results

### Timing of puberty

Figure 1 shows the distribution of menarche/initial perineal swelling and first ovulation by month (panel A) and age (panel B). Consistent with previous reports of outdoor housed rhesus monkeys (Wilson et al., 1986a; Wilson et al., 1988b), menarche occurred in every month except April and May while first ovulation was restricted to the months of Sept through March. This bimodal distribution of first ovulation resulted in 22 females ovulating

at  $31.2 \pm 1.54$  mo (range 28.1 – 34.3 mo) and 14 females ovulating at  $40.6 \pm 1.45$  mo (range 38.9 – 44.1 mo). The average duration of adolescent sterility, defined as the interval between menarche and first ovulation, was  $3.94 \pm 3.39$  mo. Age at menarche was significantly correlated with age at first ovulation ( $r = 0.72$ ,  $p < 0.001$ ). Although the duration of adolescent sterility was not correlated with age at menarche ( $r = -0.10$ ,  $p = 0.58$ ), it was positively related to age at first ovulation ( $r = 0.65$ ,  $p < 0.001$ ), suggesting females that ovulated later had a longer interval between menarche and this ovulation.

### **Dominance rank; group agonistic behaviors; and parameters of puberty**

The relative dominance rank of a female was significantly related to age at menarche ( $r=0.39$ ,  $p=0.019$ ) and age at first ovulation ( $r=0.503$ ,  $p=0.002$ ; Figure 2), but not to the duration of adolescent sterility ( $r = 0.28$ ,  $p = 0.100$ ). Thus, more dominant females tended to have an earlier onset of puberty and occurrence of first ovulation. However, dominance rank accounted for only 15% and 25% of the variance in age at menarche and first ovulation, respectively. Because dominance rank represents an animal's position in the social hierarchy that is based on which animal submits to others in a group in response to an actual or perceived aggressive act (Bernstein, 1970), the analysis of the relative dominance rank and the frequency of agonistic behaviors showed that lower dominance status was significantly related to the amount of submissive behavior directed to others in their social group ( $r = 0.60$ ,  $p < 0.001$ ) as well as more aggression received from others ( $r = 0.32$ ,  $p = 0.06$ ). Conversely, higher rates of aggression directed towards others ( $r = -0.50$ ,  $p = 0.002$ ) and higher rates of submission received from others ( $r = -0.49$ ,  $p = 0.003$ ) were related to higher dominance status. Thus, rates of agonistic behavior – both aggression and submission directed at or received from group members - rather than dominance rankings were used for the for the multiple regression analysis.

### **Predictors of menarche**

Illustrated in Table 2 are bivariate correlations between the phenotypes assessed and age at menarche/initial perineal swelling, age at first ovulation, and duration of adolescent sterility. With respect to significant correlations, earlier age at menarche was significantly related to (1) higher rates of submissive behavior received from group mates; (2) lower baseline serum concentrations of cortisol prior to the social separation test; (3) a greater percentage increase in cortisol in response to the stressor; (4) more weight gain; and (5) lower scores of Appeasement behaviors from the emotionality tests. Table 3 shows the results of the stepwise multiple regression analysis. Age at menarche was significantly predicted from the statistical combination of three factors, accounting for 58% of the variance. Examination of the  $\beta$  coefficients shows that an earlier age at menarche was predicted by the combination of (1) fewer appeasement behaviors during the HI; (2) more submissive behavior received from group mates; and (3) more weight gain during the juvenile period. Figure 3 shows the contribution of each of these three variables to explained variance in age at menarche.

Table 4 shows how these three predictors of menarche were related to other variables assessed in the study (noting that only significant correlations are listed). Submission received from others in the group, a behavior significantly related to higher dominance rank, correlated positively to the frequency of aggression directed to others in their group as well as to parameters of serum cortisol. Females that received high rates of submission had lower baseline cortisol but responded with greater increases during the social separation stressor. Appeasement behaviors during the standardized tests were negatively correlated with juvenile weight gain. While these behaviors were positively correlated with submission to others in the subjects' social group, the relation was marginally significant ( $p = 0.06$ ). Finally, juvenile weight gain was not significantly related to agonistic behavior in the social group but was positively related to the amount of affiliation received from group mates.

Although, as noted above, weight gain was negatively related to appeasement scores from the emotionality tests, both independently predicted age at menarche.

### Predictors of first ovulation

As shown in Table 2, earlier age at first ovulation was significantly related to (1) lower rates of aggression received from group mates; (2) less submissive behavior directed to others in their group; (3) higher rates of affiliation directed to others in the group; (4) a greater percentage change in cortisol to the stressor; (5) more weight gain; (6) and lower scores from the Appeasement component of the emotionality tests. Entering these variables in a multiple regression analyses revealed that the combination of five variables accounted for 71% of the variance in age at first ovulation (Table 3). Examination of the  $\beta$  coefficients shows that an earlier first ovulation was predicted by the combination of (1) receiving less aggression from group mates; (2) exhibiting fewer anxiety behaviors during the emotionality tests; (3) gaining more weight during the juvenile phase; (4) having a larger percent increase in serum cortisol during the stressor; and (5) exhibiting fewer appeasement behaviors during the emotionality tests. Figure 3 shows the contribution of each of these five variables to explained variance in age at first ovulation.

These five predictors of first ovulation were significantly related to a number of other variables (Table 4). The significant correlations of Appeasement behaviors during the emotionality tests and weight gain with other variables were described in the *Menarche* section above. Females that received more aggression from group mates correspondingly submitted more, indicative of lower dominance status, and also had significantly higher distress and vigilance scores during the emotionality tests. Furthermore, the magnitude of the acute cortisol response to a transitory stressor was inversely related to the relative rank of the (the lower the relative rank, the higher the dominance status), as well as to behaviors associated with higher dominance status, specifically a positive correlation with the frequency of submissive behaviors received from others as well as aggression directed towards others and an inverse correlation with submission towards others. The change in serum cortisol was also inversely related to baseline cortisol. The negative correlation between baseline serum cortisol and submissive behaviors received from others (Table 4) implies that females that received more submissive behavior from others (more likely more dominant females) have lower serum cortisol levels at baseline. This is also consistent with the observation that more subordinate females (having a higher relative rank ratio) have higher baseline cortisol ( $r = 0.37, p = 0.03$ ). Finally, anxiety behaviors during the emotionality tests were inversely related to the amount of affiliation received from group mates.

### Predictors of duration of adolescent sterility

Three variables showed significant correlations with the durations of adolescent sterility (Table 2). Females with a shorter interval between menarche and first ovulation received less aggression received from group mates, were less submissive to others in their groups, and showed fewer distress behaviors during the emotionality tests. The results of the multiple regression analysis showed that the statistical combination of three variables accounted for 45% of the variance in the duration of adolescent sterility (Table 3). A shorter interval between menarche and first ovulation was predicted by (1) lower rates of submissive behavior directed towards group mates; (2) lower rates of anxiety behaviors, and (3) lower rates of distress related behaviors during the emotionality tests. Figure 3 shows the contribution of each of these three variables to explained variance in the duration of adolescent sterility.

Table 4 shows these predictors of the duration of adolescent sterility were significantly related to a number of other variables. The frequency of anxiety behaviors during the emotionality tests was described above in the *First Ovulation* section. Submission towards others was, not surprisingly, positively related to the amount of aggression a female received from group mates, behaviors predictive of lower dominance status. Furthermore, rates of submission directed to others were inversely related to how much affiliative behavior females initiated. Females that showed high rates of submission also showed a lower cortisol response to the acute stressor yet higher distress behaviors during the emotionality tests. Distress scores were positively related to the amount of aggression received from group mates and how much submission these female had to emit.

## Discussion

The results of the present study indicate that, in addition to weight gain and cortisol responsivity, a number of behaviors significantly predict the timing of puberty in female rhesus monkeys. The analyses were restricted to behavioral and physiological phenotypes obtained prior to the onset of puberty, defined as menarche. Thus, variance in these measures is not a consequence of puberty but rather reflect developmental differences in the juvenile females independent of post menarchial exposure to estradiol. The approach allowed a determination of factors that predict both puberty onset and completion, defined as first ovulation, as well as the intervening interval of adolescent sterility. Together, this analysis shows that growth velocity, cortisol responsivity to an acute stressor, agonistic behavior within the social groups, and emotional reactivity, collectively predict the timing puberty, contributing to 58% of the variance in the age of menarche, 71% in age at first ovulation, and 45% in the duration of adolescent sterility accounted for by the statistical combination of these factors. Globally, our findings suggest that higher weight gain during the juvenile period, combined with lower emotional reactivity, evidenced as lower levels of anxiety, distress and appeasing behaviors in response to a potentially threatening situation, and lower aggression but more submission received from group mates predicts earlier age at menarche and first ovulation.

For the present study, puberty onset was defined as menarche or the first appearance of perineal swelling, both of which are the result of initial developmental increases in ovarian estradiol secretion (Terasawa et al., 1984; Wilson et al., 1986a). Because the nocturnal increase in gonadotropins in monkeys (Pohl et al., 1995; Terasawa et al., 1984; Wilson et al., 2003) and children (Apter et al., 1993) represent the actual onset of puberty, menarche and accompanying perineal changes denote accurate albeit somewhat later markers of puberty onset in monkeys. We defined the completion of puberty as first ovulation, an age when females are reproductively mature and capable of producing offspring but some 3 to 4 years before adult skeletal maturity is attained (Tanner and Wilson, 1990; Wilson and Tanner, 1991). While the three parameters of reproductive maturation assessed in this study are distinct, they reflect a continuum of an underlying neurobiological process characterized by a gradual acceleration in the pulsatile release of GnRH from the mediobasal hypothalamus stimulating pituitary gonadotropin secretion (Watanabe and Terasawa, 1989; Wildt et al., 1980), a process that occurs largely independent of ovarian steroid negative feedback (Chongthammakun et al., 1993; Chongthammakun and Terasawa, 1993; Pohl et al., 1995; Wilson et al., 2004a; Wilson et al., 1986b). Importantly, a change in sensitivity to estradiol negative feedback inhibition of gonadotropins (and presumably GnRH) secretion is important for the final stages of puberty, including the duration of adolescent sterility and timing of first ovulation (Rapisarda et al., 1983; Wilson, 1995). Because rhesus monkeys housed outdoors are seasonal breeders (Walker et al., 1984), season also influences the timing of first ovulation but not menarche (Wilson et al., 1986a; Wilson et al., 1988b). Menarche, representing the consequence of the initial neuroendocrine drive to the pituitary –

ovarian axis, can occur independent of season but the final stages of puberty and first ovulation are restricted to the fall and winter. If this neuroendocrine maturation occurs during the spring and summer, first ovulation is delayed until the subsequent fall (Wilson and Gordon, 1989). This differential pace of maturation of the hypothalamic – pituitary – ovarian axis accounts for the bimodal distribution of first ovulation in the present study. Thus, the factors found to influence the variance of the timing of the three parameters of puberty would do so by ultimately affecting the acceleration in GnRH pulsatility and subsequently the decrease in estradiol negative feedback inhibition.

The inclusion of weight gain in the model of predictors of menarche and first ovulation is consistent with a long-standing literature that accelerated prepubertal growth contributes significantly to an earlier onset of puberty (Frisch, 1984; Mesa et al., 2010; Tanner and Whitehouse, 1975; Wehkalampi et al., 2011). Weight gain is a surrogate measure of a number of variables, including the balance between food intake and energy expenditure; stress-induced inhibition of growth signals (Coplan et al., 2000); neuroendocrine signals that stimulate lean and bone mass; and signals from accumulating fat mass. Our study did not differentiate between bone, lean and fat mass so it is not possible to say with any confidence what compartment or signals were most important. However, several growth-related signals may be important. A previous study showed that leptin administration to juvenile female monkeys accelerates the initial rise in LH and age at menarche but the approach was unable to determine whether first ovulation was also advanced (Wilson et al., 2003). These data are consistent with emerging evidence that excess body fat in childhood results in early puberty in girls (Burt Solorzano and McCartney, 2010; Freedman et al., 2003; Reardon et al., 2009), although the role of leptin in this process is somewhat uncertain (Elias, 2012; Plant, 2008). On the other hand, manipulation of the GH – IGF-I axis in female monkeys has no effect on the age at menarche but alters first ovulation, with GH (Wilson et al., 1989) and IGF-I accelerating (Wilson, 1998) and a somatostatin analog delaying age at first ovulation (Wilson et al., 2004b; Wilson and Tanner, 1994). The effect of IGF-I was limited to the later stages of puberty, as it decreased sensitivity to estradiol negative feedback inhibition of LH (Wilson, 1995). Because both leptin (Castellano et al., 2010) and IGF-I (Hiney et al., 2009) target kisspeptin neurons in the hypothalamus, these could be the neurochemical link between the juvenile growth and the pubertal rise in GnRH.

Interestingly, juvenile weight gain was not significantly related to agonistic behavior within the group but was positively related to the amount of affiliation received, suggesting that being a target of prosocial behavior from group mates is associated with more growth and may mitigate adverse developmental effects of antagonistic social interactions (Sanchez, 2006). Because our focal animal sampling procedure for social behaviors within the group did not capture forms of social support from family members that may occur during agonistic interactions (Matheson and Bernstein, 2000), it is possible that inclusion of these behaviors would account for additional variance in outcomes associated with subordinate status. In addition, a significant predictor of earlier age at first ovulation was a greater cortisol response during the acute social separation test. While this seems somewhat counterintuitive, it could be argued that this heightened reactivity to an acute stressor may be adaptive, as it prepares the individual metabolically to recruit energy resources and to respond appropriately to the challenge (Dorn and Chrousos, 1997). This seems particularly true for individuals living in protected social environments (Boyce and Ellis, 2005), perhaps for those growing up as dominant monkeys. Indeed, a simple correlation analyses showed that lower baseline cortisol predicted greater increase in response to the acute social separation (Table 4) and an earlier age at menarche was related to lower baseline cortisol (Table 2). This greater cortisol increase co-varied with behaviors that are indicative of higher dominance status, including lower rates of submission received from others yet higher rates of aggression directed to others. Importantly, these agonistic behaviors were

included in the regression models predicting puberty timing, notably aggression received predicted later first ovulation, submission received predicted earlier menarche, and submission towards other predicted a longer adolescent sterility. It is worth noting that socially subordinate macaques (Silk, 2002) and rats (Blanchard et al., 1995) often receive random, unpredictable acts of aggression from group mates, analogous to variable stress paradigms used in other animal models (Herman et al., 1995), reflecting the strong psychosocial component needed to model to the social stress experienced by people (Anisman and Matheson, 2005; Huhman, 2006).

Although single morning (baseline) cortisol values are quite variable and often do not predict dominance status in adult females cortisol (Czoty et al., 2009; Gust et al., 1993; Stavisky et al., 2001), the present data suggest that rates of agonistic behaviors female engage in rather than dominance status per se are more informative of cortisol regulation, particularly during development. Thus, the higher basal cortisol in animals that receive less submission – which also receive more aggression and, consequently, exposed to repeated/ chronic harassment - is consistent with similar effects observed in monkeys (Clarke et al., 1998; Dettling et al., 2002a; Sanchez, 2005; Shannon et al., 1998) and children and adults exposed to early life stress (Cicchetti and Rogosch, 2001; Gunnar and Vazquez, 2001; Heim and Nemeroff, 2001; Tarullo and Gunnar, 2006). However, the directionality of the effect is not consistent throughout the literature because chronic stress can also result in basal hypofunction of the HPA axis (Fries et al., 2005). Data from a diverse series of studies in monkeys and humans do not show a clear pattern of how early social experience affects cortisol stress reactivity, as some data suggest a blunted cortisol response to an acute stressor (Barr et al., 2004; Hart et al., 1995; MacMillan et al., 2009) while others show an exaggerated response to a pharmacological challenge (Sanchez et al., 2010) or a social stressor (Bremner et al., 2003; Heim and Nemeroff, 2001; Tarullo and Gunnar, 2006). Nonetheless, the developmental regulation of the LHPA in female rhesus monkeys is poorly understood, particularly in the context of differential exposure to aggression and harassment. Studies must elucidate how exposure to high rates of aggression that requires high rates of submission disrupts the neuroendocrinology of puberty and whether signals from the LHPA axis or other stress-related neurochemicals mediate these effects.

The present analysis also provides evidence that particular aspects of a female's emotional reactivity also uniquely contribute to pubertal timing. In general, higher emotional reactivity predicted later, not earlier, age at menarche and first ovulation. Appeasement scores from the emotionality tests, which were negatively related to weight gain, contributed significantly, indicating females who submitted more in response to threatening stimuli had a later menarche and first ovulation. Similarly, higher anxiety scores also predicted later first ovulation and a longer duration of adolescent sterility. Finally, distress scores uniquely predicted a longer interval between menarche and first ovulation. Our findings are consistent with reports in humans that pubertal timing is affected by the child's reactivity to the environment and resulting anxiety (Boyce and Ellis, 2005; Ellis et al., 2011; Kim and Smith, 1998). However, the question of whether these emotional responses reflect individual traits of the animals or "states" resulting from their social history cannot be answered here. Appeasement scores positively correlated with rates of submission to others in the subjects' social group, although the relation was marginally significant ( $p = 0.06$ ), and females that exhibited lower anxiety scores were more often targets of affiliative behavior from group mates. Furthermore, distress scores were significantly predicted from the amount of aggression a female received in her group. While these associations might suggest these emotional behaviors derive from the animal's social experience and their social dominance rank, preliminary data from our group indicate that rates of aggression received from group mates is heritable, when social status is controlled for (Sanchez, McCormack, Johnson, Wilson & Rogers, unpublished). An anxious temperament (Kalin and Shelton, 2003) and

trait-like behavioral inhibition (Kalin and Shelton, 1998) are evident in monkeys using the type of tasks used in our studies and are known to be heritable (Rogers et al., 2008; Williamson et al., 2003). The expression of these behaviors involves a limbic neural circuitry, including the amygdala, bed nucleus of the stria terminalis (BNST), prefrontal cortex (Kalin et al., 2007; Kalin et al., 2005; Oler et al., 2010), that is regulated by stress-induced changes in glucocorticoids as well as limbic and hypothalamic expression of corticotropin releasing hormone (CRH) (Schulkin et al., 1998). Limbic overexpression of CRH not only increases emotionality but also disrupts ovarian cycles in rats (Keen-Rhinehart et al., 2009). What remains to be determined is how social experiences interact with innate temperament during the juvenile period to modulate this neural circuitry to alter puberty timing.

The results of the current study suggest that social stress during the juvenile period in monkeys predicts a later, not earlier, puberty onset, a finding in direct contrast to data derived from girls (Ellis and Essex, 2007; Ellis and Garber, 2000; Ellis et al., 2011; Hulanicka et al., 2001; Kim and Smith, 1998). While higher BMI was a significant predictor of earlier pubertal timing in girls, because it was not related to whether a father was absent, it was not considered an important variable in the model of father absence predicting earlier puberty (Deardorff et al., 2011). However, father absence predicted breast but not pubic hair development whereas BMI predicted both. It is possible that BMI and adipose related signals are the critical variables, regardless of the presence of the father. Importantly, from the perspective of understanding the important neurobiological mechanisms, it is unclear what signals caused by exposure to a disruptive home environment - other than those stemming from emotional feeding and excess weight gain (Epel et al., 2004) - could accelerate the maturation of the GnRH system. Prospective studies in female monkeys show that eating a high fat diet accelerates puberty (Schwartz et al., 1988; Terasawa et al., 2012), supporting the positive relation between BMI and puberty onset in girls (Frisch, 1984; Mesa et al., 2010; Tanner and Whitehouse, 1975; Wehkalampi et al., 2011). Indeed, stress reactivity is predictive of increased adiposity in boys and girls (Roemmich et al., 2007), perhaps the result of emotional feeding of high caloric foods in response to a stressful environment during development (Epel et al., 2004). Animals in the present study were fed the typical laboratory low fat, high fiber diet. Assessment of caloric intake in adult female rhesus monkeys indicates food intake is similar between dominant and subordinate females in this dietary environment whereas subordinates eat nearly twice as many calories compared to dominant females when a "typical western diet" of comprised of fat and sugar is available (Michopoulos et al., 2012c). Thus, the discrepancy between the present data combined with previous reports of social stress delaying puberty in female monkeys (Schwartz et al., 1985; Wilson et al., 1986a; Zehr et al., 2005) and the data from children growing up in stressful environments (Ellis and Essex, 2007; Ellis and Garber, 2000; Ellis et al., 2011; Hulanicka et al., 2001; Kim and Smith, 1998) may be due to diet. We predict that if subjects in the present study were fed a high caloric diet, puberty timing would be significantly advanced in adolescent females exposed to higher rates of agonistic behavior from group mates.

In summary, the results of the present study indicate that growth velocity, cortisol responsivity, agonistic behavior within their natal groups, and emotional reactivity, notably a higher incidence of anxiety-like, appeasing, and distressful behaviors to novel or perceived threatening situations, collectively alter the timing of puberty. While early puberty may be more advantageous for the individual from a fertility standpoint, it presents significant health risks, including increased the risk for a number of estrogen dependent cancers and as well as the emergence of mood disorders during adulthood (Golub et al., 2008; Walvoord, 2010). Furthermore, it is possible that increased emotional reactivity associated with delayed puberty could persist, increasing the risk for emotional dysregulation to socially challenging

situations as females progress into adulthood. A limitation of the present study is its correlational approach. Indeed, the data argue for prospective studies that will determine how emotional reactivity shown to be important for pubertal timing is affected by early social experience and temperament and how growth and stress-related signals affect the developmental increase in GnRH and gonadotropins. Moreover, a most critical question for future studies would be to assess the long-term health consequences of early versus late puberty onset in this model. Finally, because offspring assume the relative ranks of their mothers when rhesus monkeys are housed in social groups, the present study could not disentangle whether the pubertal outcomes observed were the result of the social experience of growing up subordinate, prenatal consequences of stress experienced by subordinate mothers (Wadhwa, 2005), or transgenerational transmission of these behavioral and physiological phenotypes from the mother, potentially via epigenetic mechanisms (Nelson and Nadeau, 2010). Because social status in adult female rhesus monkeys has a dramatic effect on gene expression, with a significant global association of dominance ranks and methylations profiles (Tung et al., 2012), prospective studies must determine whether these status-related differences in key signaling pathways are transmitted from the mother to the offspring and influence reproductive maturation across generations.

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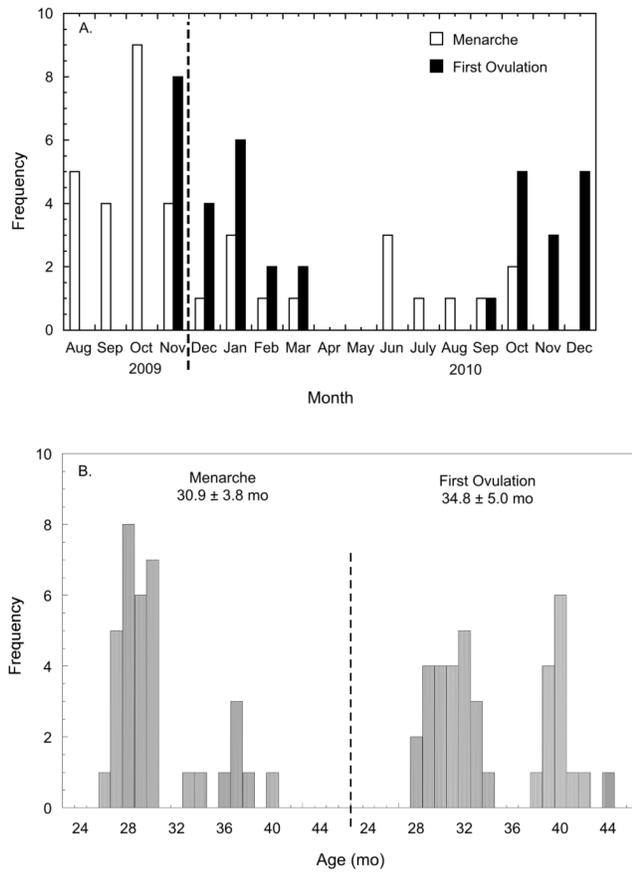
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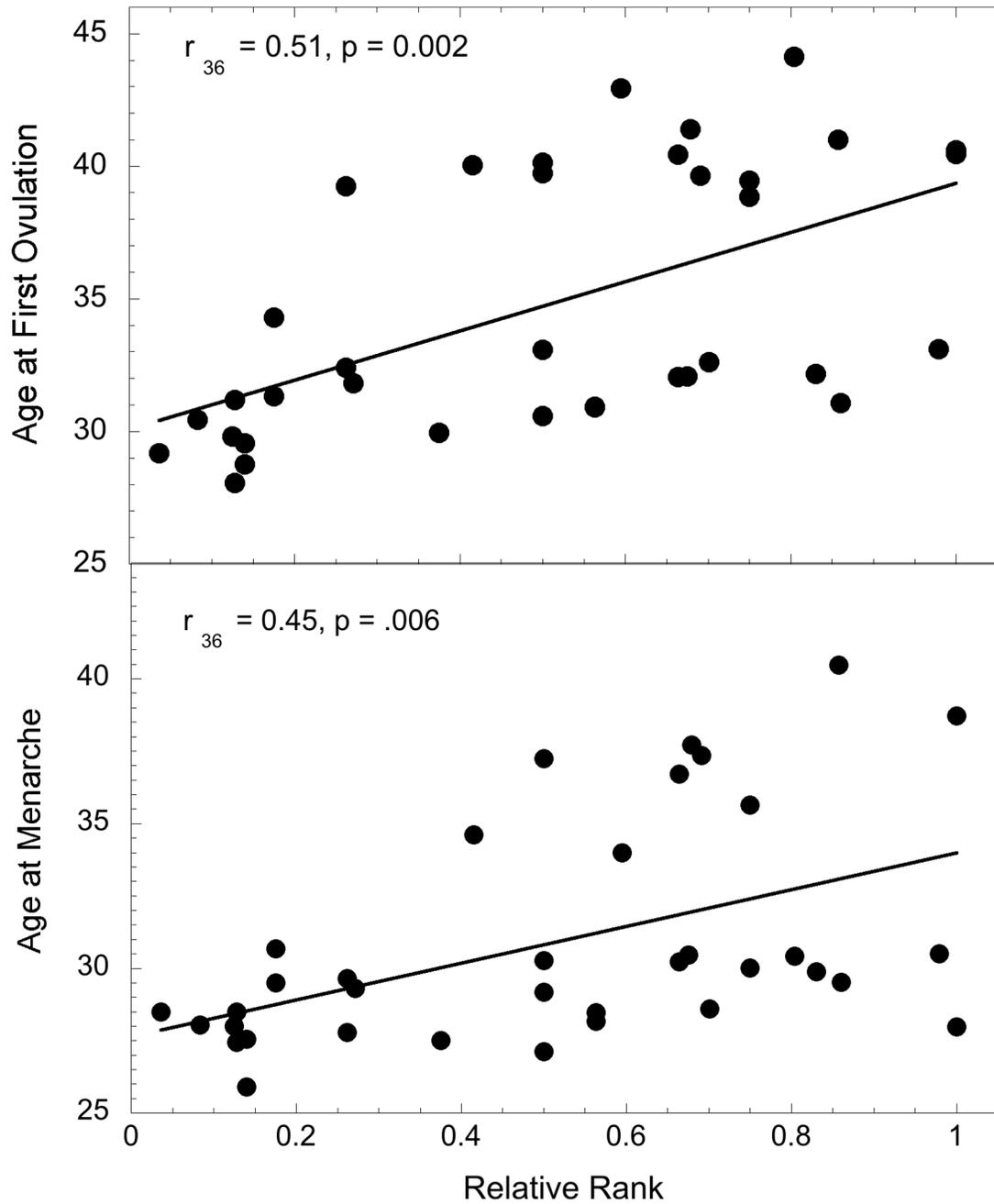
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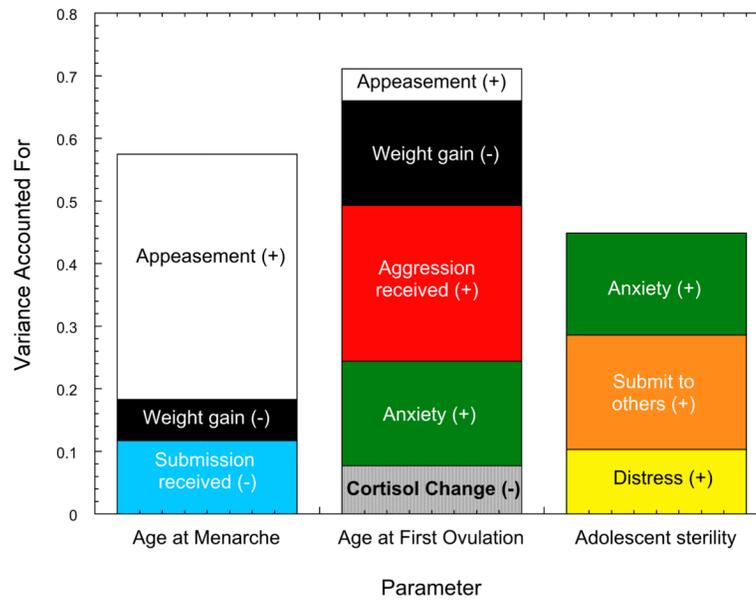
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**Figure 1.** Shown is the frequency distribution of season (panel A) and age (panel B) at menarche and first ovulation. Also indicated are the mean  $\pm$  standard deviation for age at menarche and age at first ovulation in months.



**Figure 2.** Regression of a female's relative social dominance rank (0.01 – 1.00) and age at menarche (upper panel) and first ovulation (lower panel).



**Figure 3.**

The amount of variance in age at menarche, age at first ovulation, and duration of adolescent sterility accounted for by each predictor in the multiple stepwise regression models described in Table 3. A plus (+) or minus (-) indicates the value of the  $\beta$ -coefficient predicting later puberty in the regression model

**Table 1**

Mean  $\pm$  SD as well as minimum and maximum values for behavioral and physiological phenotypes derived from the juvenile phase of development prior to menarche. For emotional behaviors, HI refers to the human intruder test and AA to the approach – avoidance test. Numbers associated with each emotional behavior are the loading scores in the principal components analysis. All behaviors for the HI occurred during the stare condition with the exception of freezing which occurred during the profile condition.

Phenotype	Mean	SD	Minimum	Maximum
<b>Social Behaviors</b>				
Aggress others	.62	0.73	.00	2.98
Receive aggression	1.24	0.80	.00	3.62
Submit to others	2.87	1.57	.00	7.21
Receive submits	.67	0.70	.00	2.74
Give affiliation	29.02	7.56	16.45	49.17
Receive affiliation	12.25	4.15	7.40	23.66
<b>Physiological</b>				
Weight gain	1.32	0.22	.87	1.74
Baseline cortisol	27.79	6.48	13.40	42.04
Post stress cortisol	43.11	8.82	27.09	68.87
Percent change in cortisol	59.9%	36.2	11.1	122.1
Activity	$13.3 \times 10^3$	$11.2 \times 10^3$	$3.19 \times 10^3$	$7.37 \times 10^4$
<b>Emotional Behavior</b>				
<u>Distress</u>				
Freeze (HI profile; -0.66)	8.17	2.10	.88	10.11
Coo (HI; 0.81)	12.18	21.03	.00	88.00
Withdraw-locomotion (AA; 0.69)	17.51	1.32	4.00	36.00
Inspect object (AA; -0.48)	5.89	3.16	.00	13.00
Coo (AA; 0.88)	2.03	7.85	.00	44.00
<u>Apppeasement</u>				
Avert gaze – look away (HI; 0.81)	25.94	15.22	1.00	70.00
Lip smack (HI; 0.69)	8.94	8.08	.00	38.00
<u>Anxiety</u>				
Scratch-self, yawn (HI; 0.75)	5.24	9.97	.00	42.00
Avert gaze-look-away (AA; 0.82)	6.20	6.93	.00	29.00
Threat object (AA; 0.85)	1.40	2.20	.00	8.00
Withdraw-locomotion (HI; 0.55)	16.18	2.17	.00	51.00
<u>Aggression</u>				
Tooth grind (HI; 0.62)	4.12	14.39	.00	74.00
Bite object (AA; 0.64)	2.31	3.16	.00	11.00
Explore Cage (AA; 0.54)	1.28	0.13	.00	3.29
<u>Vigilance</u>				
Threat (HI; -0.81)	111.12	81.48	.00	379.00

Phenotype	Mean	SD	Minimum	Maximum
Inspect object (AA; 0.53)	5.89	3.16	.00	13.00
Freeze (HI profile; -0.47)	8.17	2.10	.88	10.11

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**Table 2**

Pearson product moment correlations between phenotypes and parameters of puberty. The correlations with emotional behaviors are with the five distinct components derived from the principal component analysis of the data from the approach – avoidance and human intruder tests.

Phenotype	Menarche – swelling	First Ovulation	Adolescent sterility
<b>Social behavior and serum cortisol</b>			
Aggress others	-0.28	-0.27	-0.07
Receive aggression	0.27	0.52**	0.44**
Submit to others	0.21	0.42*	0.35*
Receive submits	-0.35*	-0.25	-0.03
Give affiliation	-0.26	-0.37*	-0.22
Receive affiliation	-0.04	-0.18	-0.22
<b>Physiological Variables</b>			
Baseline cortisol	0.37*	0.31	0.01
Post stress cortisol	-0.01	-0.13	-0.16
Percent change in cortisol	-0.34*	-0.43**	-0.22
Weight gain	-0.49**	-0.43**	-0.07
Activity	-0.13	-0.23	-0.18
<b>Emotional Behaviors</b>			
Distress	-0.05	0.21	0.37*
Appeasement	0.62**	0.45**	-0.06
Anxiety	-0.01	0.12	0.22
Aggression	0.11	0.05	-0.05
Vigilance	-0.01	0.20	0.29

\*  
p < 0.05,

\*\*  
p < 0.01

**Table 3**

Multiple stepwise regression models using factors listed in Table 1 and 2 to predict age at menarche, age at first ovulation, and the duration of adolescent sterility. The PCA scores from the emotionality tests were used as predictors rather than the individual behaviors. B is the unstandardized beta coefficient and SE standard error of this coefficient;  $\beta$  is the standardized coefficient;  $R^2$  is the measure of variability in each outcome accounted for by the predictors; and the p-value is the probability that the model would be obtained by chance.

Parameter	Factor	B	SE B	$\beta$	R <sup>2</sup>	p-value
Age at Menarche	Appearance	1.96	0.53	0.51	0.58	p < 0.001
	Submission received from others in group (reflecting more dominance)	-1.86	0.70	-0.33		
	Weight gain	-5.22	2.55	-0.28		
Age at First Ovulation	Aggression receive from others in group (reflecting more subordination)	2.90	0.68	0.49	0.71	p < 0.001
	Anxiety	2.26	0.63	0.39		
	Weight gain	-5.90	2.72	-0.26		
	Percent change in cortisol	-4.44	1.57	-0.31		
	Appearance	1.22	0.58	0.26		
Duration of adolescent sterility	Submit to others (reflecting more subordination)	0.68	0.37	0.28	0.45	p < 0.001
	Anxiety	1.89	0.57	0.47		
	Distress	1.26	0.55	0.37		

**Table 4**

Significant Pearson product moment correlations between variables that significantly predicted pubertal outcomes (Table 3) as well as with variables that did not enter the model. The sign (+ or -) of the  $\beta$  coefficients for each predictor in the multiple regression models for each outcome are shown. Note that some variables were significant predictors for more than one reproductive outcome. Grey-out cells reflect non-significant correlations.

Reproductive Outcome Variables	Predictors from Multiple Regression Models									
	Menarche (-) Submission received	Menarche (+)FO (+) Appeasement	Menarche (-)FO (-) Weight gain	FO (+) Aggression received	FO (-) Change in cortisol	FO (+)AS (+) Anxiety	AS (+) Submit to others	AS (+) Distress		
Submission received					0.47**					
Appeasement			-0.43**				0.32+			
Weight gain		-0.43*								
Aggression received							0.49**	0.38*		
Change in cortisol	0.47**						-0.39*			
Anxiety										
Submit to others		0.32+		0.49**	-0.39*			0.38*		
Distress				0.38*			-0.38*			
Give affiliation	0.43**				0.53**		-0.36*			
Affiliation received			0.34*			-0.37*				
Baseline cortisol	-0.37*				-0.58**					
Post stressor cortisol					0.44**					
Aggress others	0.85**				0.55**					
Vigilance				0.33*						
Relative rank	-0.49**			0.32+	-0.56**		0.60**			

\* p < 0.05.

\*\* p < 0.01, +p = 0.06. FO: First ovulation; AS: Adolescence Sterility. As described in Methods, the relative rank score is the ratio of a female's rank to others in the group, with a high value reflecting more subordinate status.