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The influence of bilateral neonatal lesions of the amygdala on pubertal onset and sexual behavior in female Rhesus Macaques living in social groups

By

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Doctor of Philosophy

Psychology

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M. A., Emory University, 2008

B. A., Franklin & Marshall College, 2003

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Abstract

The influence of bilateral neonatal lesions of the amygdala on pubertal onset and sexual behavior in female Rhesus Macaques living in social groups

By Shannon Brooke Zoe Stephens

Reproduction is regulated by various physiological and environmental factors, one of which is social context. Delayed first ovulation is observed in lower-ranking female rhesus macaques, in comparison to higher-ranking females, and lower-ranking females show a tight coupling of estradiol levels and sexual behavior in social groups (Zehr et al., 2005; Wallen, 1990). The brain mechanisms modulating the effects of social context on puberty onset and sexual behavior are unknown. The amygdala is one potential region of the brain modulating the effects of social context on reproduction because of its importance for behavioral responses to social information (Thompson et al., 1969; Spiteri et al., 2010) as well as its connections to the hypothalamus (Amaral et al., 1992). We examined the effects of neonatal bilateral neurotoxic amygdala lesions on pubertal timing and sexual behavior in female rhesus macaques living in social groups. At one month of age, subjects received either bilateral amygdala lesions or a sham-operated procedure. Beginning in August, 14-17 months of age, vaginal swabs and blood samples were collected to assess the age at menarche and first ovulation. Sexual behavior was examined in a pair test and in the social group. Earlier menarche and first ovulation were observed in lesioned females in comparison to control females. Based on the current study, it is not clear what, if any, effect the amygdala has on modulating social information that alters the timing of puberty. Lesioned females showed lower levels of some female-initiated sexual behaviors in the social group observations, but there was no effect of amygdala lesions on female sexual receptivity. Estradiol did not influence the rate of behavior observed in lesioned females, though some effects were observed in control females. The mechanisms by which the amygdala alters puberty onset and sexual behavior are unknown. It is possible that puberty onset occurred earlier in lesioned females as a result of earlier removal of GABA inhibition on GnRH release, resulting in earlier menarche and ultimately, earlier first ovulation. Sexual behavior differences in amygdala-lesioned females may result from decreased estradiol sensitivity, thereby influencing the modulatory relationship that estradiol can have on female sexual behavior.

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Reproduction is costly because it requires an investment of time and energy by the female to produce offspring and this investment begins with the initial acts of sexual behavior. Engaging in sexual behavior with a male is costly to the female because it is risky, particularly for low-ranking animals that risk receiving aggression from other group members for engaging in sexual behavior with the male. Pregnancy is costly to the female because the developing fetus obtains all nutrients from the mother meaning that the mother is spending at least some additional time searching for and consuming food as well as metabolizing the food for the fetus. The costs of reproduction do not end with giving birth because maternal care is required in some species until the offspring is capable of surviving on its own. Maternal care of the offspring could require the mother to spend time finding food, which she would not directly benefit from finding, for the offspring. In mammals, providing nutrients to the offspring through maternal milk requires energy from the mother for milk production. Thus, female reproduction is costly and requires a long-term investment of time and energy.

Regulation of Reproduction in Females

Due to the time and energy investment required by the female, reproduction should only occur under conditions in which survival of both the mother and the offspring are high. If there is limited food available, the mother is not receiving the nutrition needed for herself, much less any offspring, and she risks investing the time, energy and potentially her own health to produce offspring in an environment in which the offspring may not survive. In order for reproduction to occur only under optimal circumstances, it must be regulated by both internal physiological processes as well as external environmental input. Gamete supply is one physiological way to regulate

reproduction. Unlike males, females do not have an endless supply of gametes, thus her reproductive output is determined by her limited number of gametes, which is determined by the time of birth (as reviewed by Hunt & Hassold, 2008). Female reproduction is also limited by circulating gonadal hormone levels during the ovulatory cycle. In order for ovulation to occur, estradiol levels must be high enough to induce a surge in luteinizing hormone (LH), which results in ovulation (Butcher, Collins, & Fugo, 1974; as reviewed by Knobil, 1974) and the ability of the mature ovum to be fertilized by sperm. If estradiol levels do not increase, ovulation will not occur during that cycle. Thus, female reproduction is limited by estradiol levels and the ability of estradiol to produce an LH surge. Circulating gonadal hormones are also important for the expression of female sexual behavior in rats in that estradiol is required for the lordosis response (Pfaff, 1970), which allows the male to copulate with the female. In contrast to rats, nonhuman primates and humans are capable of engaging in sexual intercourse without the presence of estradiol, though sexual behavior occurs more often when estradiol levels are high (Adams, Gold, & Burt, 1978; Zehr, Maestripieri, & Wallen, 1998; Wallen, 1990; 2001). The regulation of sexual behavior by gonadal hormones couples the expression of sexual behavior with the time of ovulation which allows the mature ovum to be fertilized by sperm. Thus, female reproduction is internally regulated by gonadal hormones that influence both ovulation and sexual behavior.

Environmental factors such as seasonal changes in day length alter gonadal hormone levels and influence sexual behavior (Singh & Greenwald, 1967; Hoffman, 1968; Takahashi, Ford, Yoshinga, & Greep, 1977; Van Horn, 1980; Walker, Wilson, & Gordon, 1984), which demonstrates that environmental factors regulate reproduction.

Day length can alter estrous cycle length in rats in that longer day lengths result in a higher occurrence of irregular or five day cycles, as opposed to the typical four day cycle (Hoffman, 1968). Constant light exposure produces persistent estrus in rats characterized by constant elevation of luteinizing hormone (LH) and estradiol (Singh & Greenwald, 1967; Takahashi et al., 1977). Rhesus macaques living outdoors have a breeding season, which occurs in the fall and winter months, and regular ovarian cycles and sexual behavior occur almost exclusively during the breeding season (Van Horn, 1980; Walker et al., 1984).

In addition to these nonsocial environmental inputs that regulate reproductive processes, social context such as the presence of an unfamiliar male or group structure influence gonadal hormone levels and sexual behavior (Van der Lee & Boot, 1955; 1956; Whitten, 1956; Bruce, 1959) indicating that social context influences reproduction. Three social influences on hormonal regulation have been identified in mice: the Lee-Boot Effect, the Whitten Effect, and the Bruce Effect. Van der Lee and Boot (1955, 1956) found that female mice living in small groups had longer estrous cycles than did isolated females, meaning that the presence of other females in a female's social environment suppressed her ovarian function, making ovulation less regular and predictable. Whitten (1956) found that if a male was present in small groups of females, their ovarian cycles were of normal length and synchronized. Thus male presence in a female's social environment prevents the Lee-Boot effect and normalizes female reproductive function, a phenomenon now called the Whitten Effect. Lastly, Bruce (1959) found that the introduction of an unfamiliar male up to four days after mating with

a different male causes pregnant or pseudopregnant females to resume ovarian cycles. Thus in this case, the social conditions terminated the pregnancy.

The Lee-Boot effect (1955, 1956) is not unique to mice and has also been demonstrated in naked mole rats as well as wild rats (Sahu & Ghosh, 1982; Smith, Faulkes, & Abbott, 1997). Naked mole rats show ovarian suppression, the Lee-Boot Effect, when living in large natal colonies. This ovarian suppression is clearly an effect of social context as females begin cycling once removed from their natal group. Recent evidence demonstrates this ovarian suppression in naked mole rats requires physical social contact with the natal group (Smith et al., 1997). Wild rats showed the opposite of the Lee-Boot Effect in that females in groups showed more regular cycles than did isolated females (Sahu & Ghosh, 1982). However, female wild rats did show the Whitten effect, displaying more regular cycles when living with a male (Sahu & Ghosh, 1982). Whether this reflects a true difference between wild and domesticated rats or an acute effect of the wild rats' transition to a laboratory setting is unresolved.

Chateau and colleagues (1976) found a decrease in estrous cycle length in some female Wistar rats exposed to male urine. Individually housed females with five day estrous cycles were exposed to male urine, without having physical contact with the male, and the following estrous cycle was reduced to four days in some females (Chateau, Roos, Plas-Roser, Roos, & Aron, 1976). Social information influences gonadal hormones in both mice and rats, but the types of social information that influence gonadal hormones in rodents may differ between species.

In addition to altering gonadal hormones, social contexts specific to mating influence sexual behavior in female rats. For example, lordosis responses decrease after

females receive repeated mounts, unless the female can pace the timing of mating by leaving the male following mounts or intromissions (Bermant & Westbrook, 1966). Thus, giving her control of the timing of the mating interaction results in a markedly different pattern of sexual receptivity. Similarly, conditioned place preference, a preference for the location where mating previously occurred, develops only in females who control the timing of mating (Paredes & Vazquez, 1999). Though sexual behavior in female rats is strongly regulated by hormones (Pfaff, 1970), social context also influences when and if sexual behavior occurs, even in hormonally primed female rats.

In rhesus macaques, sexual initiation in female rhesus macaques is characterized by approaching the male, presenting her hindquarters to the male, or a handslapping in which the female nudges or taps the male with her hand (Wallen, 1990). Females approach within 20cm of a male more often during the follicular and periovulatory phases than in the luteal phase (Wallen, Winston, Gaventa, Davis-DaSilva, & Collins, 1984). Estradiol increases during the follicular phase and is highest during the periovulatory phase (Figure 1) (Wallen et al., 1984). Thus, female approach to the male and her handslapping solicitations are highest when estradiol levels are also high and are highly correlated with follicular increase in estradiol (Wallen, et al., 1984). Female sexual behavior is strongly related to her hormonal state, but rhesus macaque sexual behavior is also strongly influenced by social structure (Wallen, 1990; 2001). When a single female was paired with a single male and tested on every day of the female's ovarian cycle, some sexual behavior was observed on every day of the menstrual cycle and was weakly related to her ovarian function (Goy, 1979). By contrast, when females lived in social groups containing a single male, sexual behavior was tightly coupled to

estradiol levels and seen primarily in the periovulatory portion of her ovarian cycle (Wallen et al., 1984). When social groups contained both multiple males and multiple females, female sexual behavior was high throughout the first half of the menstrual cycle, the follicular and periovulatory phases, but low during the luteal phase (Wilson, Gordon, & Collins, 1982), as was the case with the single male, multiple female groups (Wallen et al., 1984).

In addition to social group structure, female social rank influences sexual behavior in that high-ranking females initiate sexual behavior more often than low-ranking females during the follicular phase. High-ranking females engage in high amounts of sexual behavior throughout the follicular and periovulatory phases and consequently, show no significant correlation between their estradiol levels and initiation of proximity to males during the follicular phase of the menstrual cycle (Wallen, 1990). High-ranking females do not have as much competition for access to the males and therefore, sexual initiation by high-ranking females is less tightly coupled to estradiol levels (Wallen, 1999). In contrast to high-ranking females, low-ranking females closely approach adult males at high rates only around the time of their estradiol peak and thus, behavior is strongly correlated with changes in estradiol (Wallen, 1990). For a low-ranking female, competing with a high-ranking female to get close to a male is risky and only occurs when female sexual motivation is at its highest, when estradiol levels are high (Wallen, 1999). Taken together these findings demonstrate that the relation between hormonal changes and female sexual behavior is strongly affected by social context.

Social rank also affects gonadal hormone regulation in that higher-ranking females more often having regular menstrual cycle lengths than do lower-ranking females

who show a higher incidence of irregular or anovulatory cycles than do higher-ranking females (Walker, Gordon, & Wilson, 1983). Thus, social rank affects both sexual behavior and gonadal hormone regulation in rhesus macaques.

Regulation of Pubertal Onset in Females

Puberty is the transition period between juvenile and adult life stages marked by the maturation of the hypothalamic-pituitary-gonadal (HPG) axis, initiated by the release of GnRH from the hypothalamus in monkeys (as reviewed by Plant, 2001) and the maturation of positive feedback mechanisms in rats (as reviewed by Döcke, 1981). In addition to these changes in gonadal hormone levels, pubertal onset is influenced by social factors. For example, the presence of a novel adult male accelerates pubertal onset in female mice (Vandenbergh, 1974) and pubertal onset is delayed in subordinate female marmosets living with a dominant female (Barrett, Abbott, & George, 1990). This influence of social context on sexual maturation demonstrates that age at sexual maturation is somewhat plastic and the timing of sexual maturation can be accelerated or delayed depending on the social cues present. In nonhuman primates, social rank is a critical social factor regulating pubertal onset. For example, in female baboons social rank influenced pubertal development such that dominant females had earlier menarche than lower-ranking females (Bercovitch & Strum, 1993). In rhesus macaques living in large age-graded social groups, social rank did not predict age at menarche, but was related to age at first ovulation with high-ranking females ovulating earlier. The mechanism of this effect of social rank on earlier pubertal onset is unknown. However, females ovulating earlier, regardless of social rank, also had higher body weights and a higher body mass index (BMI), suggesting dominant individuals enter puberty earlier due

to their greater body weight (Zehr, Van Meter, & Wallen, 2005). However, it is as likely that both earlier puberty and greater weight are both a consequence of higher rank and that weight per se is not the cause of the earlier puberty in higher ranking females.

In female marmosets, a new world primate, dominant females suppress puberty and ovulation in subordinate females and this suppression continues as long as the subordinate female is exposed to the scent of the dominant female (Barrett et al., 1990). This ovulation suppression did not reflect increased stress in the subordinate female, as circulating levels of cortisol were not higher in subordinate females (Abbott, McNeilly, Lunn, Hulme, & Burden, 1981). Thus, these results show a direct influence of social context on neuroendocrine function. Other new world monkeys show a different pattern of social influence on neuroendocrine function. In tamarins, females living with their family reached puberty between 15 and 17 months of age (Ziegler, Savage, Scheffler, & Snowdon, 1987). However, removal of the female from the family unit at nine months of age followed by exposure to an unfamiliar male accelerated pubertal onset (Tardif, 1984; Ziegler et al., 1987). Removal from the family group by itself does not induce puberty in tamarins and exposure to a novel male before puberty is necessary (Ziegler et al., 1987). Social rank and social group composition influence the age of pubertal onset and neuroendocrine function in New World monkeys by regulating gonadotropin releasing hormone (GnRH) release. Although these effects demonstrate strong influences of social context on reproductive function, the exact mechanisms by which social information affects reproductive function are unknown. It is likely that there are specific brain regions or circuits which transduce social information to affect both behavior and neuroendocrine function.

The Amygdala and Integration of Social Information

One candidate brain region for such transduction is the amygdala. This brain region, consisting of multiple nuclei, is important for social recognition, integrating social information, and the expression of species typical behavior in response to social information (Rosvold, Mirsky, & Pribram, 1954; Thompson, Schwartzbaum, & Harlow, 1969; Kling & Cornell, 1971; Petrulis & Johnston, 1999; Bennett, Greco, Blasberg, & Blaustein, 2002; Spiteri, Musatov, Ogawa, Ribeiro, Pfaff, & Agmo, 2010). In addition the amygdala is intimately connected to regions of the brain involved in neuroendocrine control, such as areas of the hypothalamus (as reviewed by Amaral, Price, Pitkänen, & Carmichael, 1992; as reviewed by Pitkänen, 2003). Lastly, the amygdala is connected and influences pathways generally regarded as influencing reward (see Baxter & Murray, 2002 for review). Sitting at the nexus of these three systems, social recognition, neuroendocrine function, and reward, the amygdala is a prime candidate for integrating social context with neuroendocrine function and sexual behavior.

Social recognition in animals is important for producing an appropriate behavioral response when interacting with other individuals. For example, a low-ranking female rhesus macaque must be able to recognize a high-ranking female walking by and respond appropriately by moving out of its way. Failure to recognize this female as high-ranking and the subsequent failure to move aside would likely result in the low-ranking female receiving aggression. Social recognition is important for producing species appropriate behaviors during social interactions. Studies with hamsters and rats demonstrate the importance of the amygdala in social recognition. In female rats, there was greater neuronal activation in the medial amygdala (MeA) following exposure to unfamiliar male

bedding in comparison to clean bedding (Bennett et al., 2002), which shows the MeA responds to unfamiliar social odors. Examination of neuronal activity in the MeA following exposure to a familiar social odor was not completed in this study and thus, from these results it is not clear whether this increased neuronal activation in the MeA is specific to unfamiliar social odors or if the effect would also be found with familiar social odors. Elimination of estrogen receptor α (ER α) in the MeA using a viral vector against the ER α gene in adult female rats prevented social recognition of nonrelated juvenile rats even after repeated exposures (Spiteri et al., 2010). Control female rats investigated the juvenile by sniffing its test cage and investigations occurred significantly less often in the second, third and fourth testing sessions in comparison to the first session. A second novel juvenile was presented during the fifth testing session and the number of investigations by the control female increased and was no different than the behavior shown during the initial exposure to the first juvenile. In female rats lacking ER α in the MeA, the number of investigations of the juvenile rat during the first testing session did not differ from control females, however the number of investigations did not decrease after repeated exposure to the same juvenile. Females lacking ER α in the MeA failed to recognize the juvenile as familiar and consistently behaved as if the juvenile presented was a novel juvenile. When a second novel juvenile was introduced, females lacking ER α in the MeA showed the same level of investigatory behavior as the previous four testing sessions demonstrating that females lacking ER α in the MeA treated all juveniles as unfamiliar regardless of any previous experience with juvenile (Spiteri et al., 2010). The findings from both of these studies demonstrate that the MeA is important for processing social information in female rats and ER α in the MeA is important for social recognition.

In hamsters, the preference for male odors over female odors was eliminated with amygdala lesions despite still having the ability to differentiate between male odors (Petrulis & Johnston, 1999). Thus discrimination was intact, but amygdala damage eliminated preference, a process requiring greater integration of experience than simple discrimination. The amygdala may influence social recognition and opposite-sex odor preference possibly by providing the animal with a value or meaning of the social stimuli. Similar studies have not been done in monkeys, however lesions of the amygdala resulted in a change in social status in some animals as well as decreased levels of aggression, social grooming, and proximity to others (Rosvold et al., 1954; Thompson et al., 1969; Kling & Cornell, 1971). The fall in social status likely results from decreased aggression and affiliative behavior by lesion males towards other animals in the group because amygdalectomized animals no longer integrated social information with their behavior. This evidence demonstrates that the amygdala is involved in processing social information as well as influencing behavioral outcomes requiring the integration of social information.

The amygdala is important for processing and integrating social information (Rosvold et al., 1954; Thompson et al., 1969; Kling & Cornell, 1971; Petrulis & Johnston, 1999; Bennett et al., 2002; Spiteri et al., 2010) and therefore, it is important to understand how the amygdala influences sexual behavior, gonadal hormone regulation, and pubertal onset to determine whether the amygdala is capable of integrating social cues that affect sexual behavior and physiology. This introduction begins by reviewing the neuroanatomy of the amygdala and the hypothalamus and the connections between these two structures that would allow for the amygdala to affect sexual behavior, gonadal

hormone regulation, and pubertal onset. Data regarding the influence of the amygdala on sexual behavior following adult lesions of the amygdala is presented for both rats and rhesus macaques. One specific aim of this study was to examine the influence of neonatal amygdala lesions on sexual behavior in rhesus macaque females living in large social groups because there is currently no data on this topic. The influence of the amygdala on gonadal hormone regulation is first reviewed in animals receiving amygdala lesions as adults followed by data on the onset of puberty following pre-pubertal lesions of the amygdala to demonstrate differences in amygdala lesion effects based on the timing of the lesion in rats. There is limited research available on the influence of the amygdala on gonadal hormone regulation and pubertal onset in female rhesus macaques and no effect of amygdala lesions was found (Spies, Norman, Clifton, Ochsner, Jensen, & Phoenix, 1976; Norman & Spies, 1981). Considering the data in rodents as well as the methodological constraints of the rhesus macaque studies, the second specific aim of this study was to examine the timing of pubertal onset in female rhesus macaques with neonatal amygdala lesions.

Neuroanatomy of the Hypothalamus and the Amygdala

The hypothalamus is important for sexual behavior and gonadal hormonal regulation in adults as well as pubertal onset in juveniles. This section briefly describes the areas of the hypothalamus important for these processes as well as offering a brief overview of the amygdala and its connections to the hypothalamus.

There are many ways of dividing the regions of the hypothalamus and this introduction focuses on a limited subset of nuclei: the arcuate nucleus (ARC), the medial preoptic area (mPOA), and the ventromedial hypothalamus (VMH). The mPOA and the

ARC are both important for hormonal regulation in rats, whereas in monkeys only the ARC regulates gonadal hormones (Velasco & Taleisnik, 1971; Kawakami, Terasawa, Kimura, & Wakabayashi, 1973; Krey, Butler, & Knobil, 1975; Gallo & Osland, 1976; Plant, Krey, Moossy, McCormack, Hess, & Knobil, 1978). In both rats and monkeys, the VMH plays an integral role in the expression of female sexual behavior (Mathews & Edwards, 1977; Mathews, Donovan, Hollingsworth, Hutson, & Overstreet, 1983; Oomura, Aou, Koyama, Fujita, & Yoshimatsu, 1988; Yahr & Greene, 1992).

The amygdala is located in the medial temporal lobe and consists of thirteen nuclei (as reviewed by Amaral et al., 1992; as reviewed by Pitkänen, 2003). This paper focuses on the medial (MeA) and lateral (LA) nuclei of the amygdala as these are of primary importance in relation to sexual behavior in female rats. The MeA and LA have direct connections to the VMH (as reviewed by Pitkänen, 2003), which would allow them to directly influence sexual behavior in female rats by influencing the VMH. With regard to gonadal hormone regulation in female rats, the available evidence focuses on the influence of the MeA, CoA, central (CeA), basolateral (BlA), and LA nuclei. In rats, the MeA, CoA, and CeA have projections to the mPOA, whereas the CoA and has lighter projections to the mPOA (Figure 2). The MeA, CeA, and CoA have light projections to the ARC (as reviewed by Pitkänen, 2003). Since the mPOA and ARC are involved with LH positive feedback of the HPG axis (Velasco & Taleisnik, 1971; Kawakami et al., 1973), the MeA, CeA, and CoA have the potential to directly influence LH positive feedback by influencing the mPOA or the ARC. Neither the BlA nor the LA has a direct projection to the mPOA or the ARC (as reviewed by Pitkänen, 2003) and therefore, the BlA and the LA should not directly affect LH positive feedback of the HPG axis.

Research in female monkeys has manipulated the whole amygdala and not focused on specific nuclei (Erikson & Wada, 1970; Spies et al., 1976). Thus it is not yet possible in monkeys, to attribute influences of the amygdala on sexual behavior and gonadal hormones to a precise region of the amygdala. In addition, there are currently no data showing direct connections between the amygdala and the ARC or the VMH however, in monkeys there has been little examination of the efferent projections from the amygdala to the hypothalamus. Thus, it is not possible to rule out that the amygdala could influence gonadal hormones or sexual behavior in monkeys based on neuroanatomical connections.

Connections between the amygdala and the hypothalamus are via two separate pathways: the stria terminalis (ST) and the ventral amygdalofugal pathway (VAF) (Cowan, Raisman, & Powell, 1965; as reviewed by Amaral et al., 1992). All five amygdala nuclei discussed in this paper project to the hypothalamus via the ST, while the BlA and LA also connect to the hypothalamus via the VAF (Cowan et al., 1965). Thus, the connections between the amygdala and the hypothalamus would allow for social information processed by the amygdala to directly affect regions in the hypothalamus important for sexual behavior and gonadal hormone regulation.

The Effect of the Amygdala on Female Sexual Behavior

Sexual behavior is strongly coupled to circulating hormone levels in that estrogen is required for the expression of sexual behavior in female rats (Pfaff, 1970). Estrogen is important for female initiated sexual behavior in rhesus macaques even though they are physically capable of engaging in sex without any hormonal input (Zehr et al., 1998; Wallen, 1990; 2001). If the amygdala influences hormonal regulation, it is possible the

amygdala would affect sexual behavior by altering gonadal hormones or it could affect sexual behavior through direct actions of gonadal hormones on amygdala function. Research in adult rats controlled for the effect of the amygdala on hormonal regulation by administering estradiol benzoate (EB) or EB and progesterone to ovariectomized rats, precluding endogenous variation in gonadal steroids. By contrast, in rhesus macaques there is only a single study of female sexual behavior and it used intact females with functional ovaries. However, amygdala lesions did not alter measured levels of gonadal hormones and therefore, any changes in sexual behavior resulting from amygdala lesions were not the result of altered levels of gonadal hormones (Spies et al., 1976). The following studies are therefore testing sexual behavior when estrogen levels are high and sexual behavior should be maximally expressed in order to understand the influence of the amygdala on sexual behavior.

Sexual Behavior in Adult Female Rats

The ventromedial hypothalamus (VMH) is required for the expression of sexual behavior as unilateral or bilateral lesions of this area decreased female sexual receptivity to the male (Mathews & Edwards, 1977; Mathews et al., 1983; Yahr & Greene, 1992). The LA and MeA all project to the VMH via the stria terminalis and the LA also projects to the VMH via the ventral amygdalofugal (VAF) pathway (as reviewed by Pitkänen, 2003), allowing these three nuclei to directly influence sexual behavior by influencing the VMH.

In the following studies in adult rats, all behavioral tests were completed by placing the female in a testing area with one male rat unless otherwise noted. All female subjects and stimulus males used were previously tested to ensure only sexually receptive

females and males were used in the experiment. Female behaviors including ear wiggling, hopping, darting, and approach to males were examined to assess female sexual motivation and interest in the male (Guarraci, Megroz, & Clark, 2004; Kondo & Sakuma, 2005). Lordosis is a reflex posture in the rat that allows the male to properly mount the female and was used as a measure of female receptivity. Lordosis quotient (LQ) is a measure of female receptivity that controls for male behavior and is the number of times lordosis is shown x 100/ number of mounts shown (Mathews & Edwards, 1977; Mascó & Carrer, 1980; Mathews et al., 1983; Guarraci et al., 2004; Kondo & Sakuma, 2005).

Influence of the Lateral Amygdala (LA) on Sexual Behavior in Female Rats

Bilateral radiofrequency lesions of the anterior LA significantly increased LQ when females received a threshold dose of EB given prior to testing (Mascó & Carrer, 1980). Lesions of the anterior LA increased LQ when an EB dose of 2.5µg/kg BW was given, but not when 5.0µg EB/kg BW was used (Mascó & Carrer, 1980). Thus, lesions of the anterior LA increased the sensitivity of the female to estradiol for inducing sexual receptivity. Given that the 5.0µg dose of EB increased LQ in all animals (Mascó & Carrer, 1980), it is likely the larger dose resulted in a ceiling effect thereby eliminating any differences between females with anterior LA lesions and control females.

Stimulation of the anterior LA should decrease LQ because lesions of the anterior LA increased LQ when given a small dose of EB ((Mascó & Carrer, 1980). However, electrochemical stimulation of the anterior LA did not affect LQ and stimulated animals showed LQs similar to sham-operated animals (Mascó & Carrer, 1980). LQ did not differ between intact and sham-operated control animals demonstrating lowering of the electrode into the amygdala did not have a damaging

effect on sexual behavior. LQ did not change following stimulation in the anterior LA which does not support the inhibitory effect of the anterior LA demonstrated with lesions of this area, but stimulation was only given with a very large dose of EB, 10µg/kg BW. A dose of EB half this large eliminated the difference in LQ between rats with anterior LA lesions and sham-operated controls (Mascó & Carrer, 1980). Therefore, it is likely a dose of 10µg EB/kg BW prior to anterior LA stimulation was too large a dose to allow detection of any inhibitory effects of LA stimulation on sexual receptivity. The anterior LA inhibits female sexual receptivity to the male in the presence of smaller doses of estrogen, but higher doses of estrogen can overcome this inhibitory effect of the anterior LA (Mascó & Carrer, 1980).

Similar to the lesion effects of the anterior LA, lesions of the posterior LA increased LQ showing the posterior LA, like the anterior LA, has an inhibitory influence on female sexual receptivity (Mascó & Carrer, 1980). Smaller dosages of EB, 1.25µg/kg BW and 2.50µg/kg BW, significantly increased LQ in rats with posterior LA lesions in comparison to sham-operated animals. Interestingly, sham-operated animals did not show lordosis at the smaller dosage of EB, 1.25µg/kg BW, whereas animals with lesions of the posterior LA did show lordosis (Mascó & Carrer, 1980). Therefore, the posterior LA has an inhibitory effect on female sexual receptivity by reducing the sensitivity to EB and sensitivity to EB is increased when the LA is removed.

The inhibitory effects of the posterior LA are also supported by data from stimulation studies (Mascó & Carrer, 1980). Electrochemical stimulation in the posterior LA following a smaller dose of EB, 2.5µg/kg BW, resulted in decreased LQ at 180 minutes after stimulation in comparison to the pre-stimulation LQ. Although LQ

decreased at 90 minutes after stimulation, this decrease in the LQ was not significant until 180 minutes after stimulation (Mascó & Carrer, 1980). Since LQ are lower in control animals at smaller doses of EB, the longer time interval following stimulation may be required to produce an inhibitory effect strong enough to produce a significant decrease in LQ. Similar to the smaller dose of EB, a larger dose of EB, 10ug/kg BW, followed by stimulation in the posterior LA produced a decrease in LQ. This decrease in LQ was significantly lower at both 90 and 180 minutes after stimulation in comparison to the LQ prior to stimulation. Unlike the smaller dose of EB, the larger dose of EB produced a significant decrease in LQ at 90 minutes after stimulation (Mascó & Carrer, 1980). The significant decrease in LQ at 90 minutes following stimulation in the posterior LA was likely due to the higher rate of lordosis observed prior to stimulation. A higher dose of EB, 10ug/kg BW, combined with stimulation in the posterior LA produced a decrease in LQ, whereas this same dose of EB did not result in a decrease in LQ following stimulation in the anterior LA (Mascó & Carrer, 1980). Thus the posterior LA has a stronger inhibitory influence than the anterior LA because a decrease in LQ can be produced even when given a large dose of EB. The posterior LA, like the anterior LA, has an inhibitory effect on female sexual receptivity and the amount of EB administered affects the magnitude of this effect.

Influence of the Medial Amygdala (MeA) on Sexual Behavior in Female Rats

Lesions of the anterior or posterior LA increase LQ, whereas bilateral lesions of the anterior MeA decrease LQ in female rats. This decrease in LQ reflects a decrease in female sexual receptivity that is specific to lesions of the anterior MeA as bilateral lesions of the posterior MeA were without effect (Mascó & Carrer, 1980). It is unlikely that the

differences between the anterior and posterior MeA reflect differences in estradiol sensitivity as two doses of EB were used, 5µg/kg BW or 10µg/kg BW, both of which have previously been shown to be above threshold and are supraphysiological doses for the induction of sexual receptivity. Sham-operated control rats had electrodes lowered into the amygdala but no current was passed through the electrode. LQ did not differ between intact and sham-operated control animals demonstrating lowering of the electrode into the amygdala did not have a damaging effect on sexual behavior (Mascó & Carrer, 1980). Lesions of the anterior MeA inhibit female sexual receptivity indicating the MeA facilitates sexual receptivity and data from stimulation studies supports this facilitatory influence of the MeA.

Stimulation in the anterior MeA increased LQ at both 90 and 180 minutes after stimulation in comparison to the pre-stimulation LQ when a dose of 2.5µg EB/kg BW was used indicating that activation of the MeA facilitates sexual receptivity (Mascó & Carrer, 1980).

Repeated behavioral testing at multiple times cannot explain this increase in LQ following stimulation in the MeA because sexual receptivity in sham-operated controls was comparable at both time intervals. When larger doses of EB, 10µg EB/kg BW, are administered stimulation in the MeA does not further increase LQ at either 90 or 180 minutes following stimulation. LQ was higher for all animals when administered 10µg EB/kg BW than when administered 2.5µg EB/kg BW. Therefore, it is likely that the higher estradiol dose produced a ceiling effect so that stimulation in the anterior MeA could not further increase the LQ (Mascó & Carrer, 1980). In summary, lesions of the anterior MeA decreased female sexual receptivity whereas stimulation of these areas

increased sexual receptivity (Mascó & Carrer, 1980) indicating that the anterior MeA facilitates sexual receptivity in female rats.

Unlike the facilitatory influence of the anterior MeA, the posterior MeA does not facilitate female sexual receptivity (Mascó & Carrer, 1980). If the posterior MeA facilitated female sexual receptivity, LQ should decrease following a lesion of the MeA. However, posterior MeA lesions did not significantly reduce LQ in comparison to sham-operated controls at either EB dose (5µg/kg BW or 10µg/kg BW) indicating that the posterior MeA does not facilitate female sexual receptivity (Mascó & Carrer, 1980). Possibly, as with lesions of the anterior LA, the posterior MeA inhibits female sexual receptivity, but this inhibition is not observable because of the large doses of EB employed in this study (Mascó & Carrer, 1980). If the posterior MeA inhibits female sexual receptivity, then stimulation in this area should produce a decrease in LQ. The effect of stimulation in the posterior MeA on sexual receptivity has not been examined and thus, the influence of the posterior MeA on sexual receptivity is unclear. It is also possible the posterior MeA has no influence on female sexual receptivity, in which case stimulation in this area should have no effect on LQ. Without complementary stimulation data, data from lesion studies is difficult to evaluate with regard to the effects of the posterior MeA on sexual receptivity.

All of the behavioral test performed by Mascó and Carrer (1980) were completed in a small open testing area without barriers where the female was unable to control her distance to the male. Sexual receptivity decreases following repeated mounting if the female is unable to pace the mating, or control the timing of mating interactions by leaving and approaching the male (Bermant & Westbrook, 1966). It is

unlikely that changes in LQ following brain manipulations in females resulted from repeated mounting, since each behavioral test consisted of ten mounts and repeated testing in sham-operated controls did not change LQ between testing sessions (Mascó & Carrer, 1980). In summary, when the female cannot pace mating, the anterior and posterior LA inhibit female sexual receptivity with the posterior LA having a stronger effect than the anterior LA. In contrast to the LA, the anterior MeA facilitates female sexual receptivity (Mascó & Carrer, 1980).

Repeated mating tests, multiple mating opportunities with periods of rest in between every ten mounts, can increase LQ and the MeA is important for this increase in LQ (Rajendren & Moss, 1993). Only females showing a LQ of at least 0.5 after the fourth testing session were used in the study to ensure that all females were sexually receptive after four repeated tests. Sham-operated controls showed an increase in LQ during the third and fourth testing session in comparison to the first session as did females with lesions of the MeA (Rajendren & Moss, 1993). Because the LQ increased with repeated mating tests in females with lesions of the MeA, the MeA is not required for an increase in sexual receptivity following repeated mating. However, the rate of increase of LQ was much lower for females with lesions of the MeA in comparison to controls (Rajendren & Moss, 1993), which demonstrates that the MeA is important for the magnitude of the increase in LQ following repeated mating tests.

All of the studies on sexual behavior thus far have focused on LQ, a measure of female sexual receptivity, but female sexual receptivity is not the only component of sexual behavior. Sexual behavior was historically divided into two classes of behavior: appetitive and consummatory behaviors. Appetitive behaviors are behaviors that increase

the probability of satisfying a need and with regard to sexual behavior would include finding a mate and behaviors that would arouse or interest the mate. Consummatory behaviors are the act of satisfying that need or in this case, the act of mating. Because sexual behavior in males and females is intimately intertwined with behavioral responses of one sex affecting the response of the opposite sex, Beach (1976) proposed the use of alternate terminology for studying sexual behavior: proceptivity and receptivity. Proceptivity is defined as the "appetitive activities shown by females in response to stimuli received from males", whereas receptivity is the female's response to be mounted, the act of mating, which is a consummatory behavior (Beach, 1976). Though female sexual receptivity is observed in many environments, female proceptive behaviors are typically limited to situations in which the female can pace the mating (Pfaus, Smith, & Coopersmith, 1999).

In a paced mating paradigm in which females can leave the male after being mounted, bilateral radiofrequency lesions or ibotenic acid lesions of the MeA did not alter receptive behaviors or proceptive behaviors such as hopping, darting, or ear wiggling (Guarraci et al., 2004; Kondo & Sakuma, 2005). Female rats pace mating events with a male by exiting the male area after being mounted. Paced mating is tested by providing an area in which only the female can enter in addition to an area containing the male rat. When females could pace mating by leaving the male area, proceptive behaviors, including ear wiggling and hopping and darting, and LQ did not differ between lesion and sham-operated ovariectomized rats primed with both EB and P. Thus, when females control the timing of mating, lesions of the MeA have no effect on proceptive or receptive sexual behaviors (Guarraci et al., 2004; Kondo & Sakuma, 2005).

In summary, the facilitatory influence of the MeA on LQ differs based on social contexts in that the MeA facilitates receptivity when the female cannot control the timing of mating, but when the female can control the timing of mating the MeA has no effect (Mascó & Carrer, 1980; Guarraci et al., 2004; Kondo & Sakuma, 2005). Therefore, the MeA is important in facilitating female sexual receptivity in situations that may not result in maximum frequency of sexual behavior.

In addition to facilitating female sexual receptivity in certain contexts (Mascó & Carrer, 1980; Rajendren & Moss, 1993), the MeA is important for assessing the social or sexual value of particular olfactory cues and ultimately, establishing a preference for intact males. Female rats prefer the odor of intact male rats rather than the odor of castrated males when there is no physical contact with the male, but lesions of the MeA eliminate this female preference for an intact male rat's odor (Kondo & Sakuma, 2005). Sham-operated rats, in which electrodes were lowered into the MeA, spent significantly more time nose-poking, an investigatory behavior, in the area containing an intact male rather than the area containing a castrated male. Radiofrequency lesions of the MeA diminished the preference for intact male rat odors such that lesion-females spent equal amounts of time nose-poking in the areas of the intact and castrated male (Kondo & Sakuma, 2005). These results demonstrate that the MeA is important for the development of a species typical odor preference of intact males.

Summary of the Amygdala and Sexual Behavior in Female Rats

In summary, the anterior and posterior LA inhibits female sexual receptivity and the anterior MeA facilitates sexual receptivity when females do not control the timing of mating (Mascó & Carrer, 1980; Rajendren & Moss, 1993). If the female can

control the timing of mating, the MeA has no effect on sexual receptivity or proceptivity (Guarraci et al., 2004; Kondo & Sakuma, 2005). Lastly, the MeA is important for the development of species specific preferences related to mating behavior (Kondo & Sakuma, 2005). Thus, the MeA and LA are two regions of the amygdala that have the potential to integrate social information and influence sexual behavior. Research in adult rats controlled for the effect of the amygdala on hormonal regulation by administering estradiol benzoate (EB) or EB and progesterone to ovariectomized rats, precluding endogenous variation in gonadal steroids. Therefore, the effects of the MeA and LA on sexual behavior in female rats are independent of any effects of the amygdala on gonadal hormone regulation.

Two pathways that connect the amygdala to the hypothalamus are the stria terminalis (ST) and the ventral amygdalofugal (VAF) pathway (as reviewed by Pitkänen, 2003). In order to examine the effects of each pathway on sexual behavior, sexual receptivity was tested following bilateral transection of either the ST or the VAF and stimulation in either the MeA or LA. Sexual receptivity was tested by measuring the LQ of ovariectomized rats primed with EB and P when placed in a testing area with one male.

Stria Terminalis (ST)

The anterior MeA facilitates sexual behavior, whereas the anterior and posterior LA inhibits sexual behavior (Mascó & Carrer, 1980; Rajendren & Moss, 1993). One pathway connecting the MeA and the LA to the hypothalamus is the ST (as reviewed by Pitkänen, 2003). The ST is an important pathway involved in the stimulatory effects of the MeA and inhibitory effects of the LA. Stimulation in the anterior MeA increases the

LQ in hormonally primed rats however, if the MeA is stimulated and the ST is bilaterally transected then female rats show no significant increase in sexual behavior (Mascó & Carrer, 1984). The facilitatory influence of the MeA reaches the hypothalamus via the ST. The ST is also important for the inhibitory effects of the LA on sexual behavior. When the LA is stimulated there is a decrease in the LQ following stimulation. If the LA is stimulated and the ST is bilaterally transected, ovariectomized rats primed with EB and P show no decrease in the LQ (Mascó & Carrer, 1984). Thus, the LA inhibits sexual behavior only if the ST is intact. Bilateral transection of the ST without amygdala stimulation did not affect the LQ in ovariectomized rats primed with EB and P in comparison to non-operated control females (Mascó & Carrer, 1984). Therefore, transection of the ST has no independent effect on sexual behavior. In summary, the ST itself does not influence sexual behavior, but the ST is influential in mediating the stimulatory effects of the MeA and the inhibitory effects of the LA on sexual behavior.

Ventral Amygdalofugal Pathway (VAF)

The ventral amygdalofugal (VAF) pathway is another pathway besides the stria terminalis that connects the amygdala to the hypothalamus (as reviewed by Pitkänen, 2003). In the MeA, stimulation increases sexual receptivity, but if the VAF is bilaterally transected and the MeA is stimulated, sexual receptivity doesn't increase following stimulation (Mascó & Carrer, 1984) indicating the VAF mediates the facilitatory effects of stimulation in the MeA. Since the MeA does not connect to the hypothalamus via the VAF (Cowan et al., 1965), it is interesting that transection of the VAF prevents the increase in sexual receptivity following stimulation in the MeA. This effect suggests the

MeA has indirect effects on the hypothalamus by influencing other amygdala nuclei, such as the BlA or LA that connect to the hypothalamus via the VAF pathway.

In contrast to stimulation in the MeA, stimulation in the LA decreases LQ (Mascó & Carrer, 1984). When the VAF is bilaterally transected and the LA is stimulated, there is a still a decrease in LQ. This decrease in LQ is comparable to the response observed in rats with an intact VAF that received ECS in the LA which demonstrates stimulation in the LA does not decrease LQ by influencing the hypothalamus via the VAF (Mascó & Carrer, 1984). Since transection of the VAF does not influence the decrease in LQ following LA stimulation, then the MeA probably does not alter LQ via the VAF by influencing the LA, but possibly by influencing the BlA. Therefore, the VAF is an important pathway for the facilitatory effects of the MeA on sexual behavior, but the VAF is not involved in the inhibition of LQ following LA stimulation.

In summary, the anterior MeA, like the VMH, can facilitate sexual receptivity in contexts when the female cannot control the timing of mating (Mathews & Edwards, 1977; Mathews et al., 1983; Mascó & Carrer, 1984; Yahr & Greene, 1992) and the MeA facilitates sexual receptivity via the ST and indirectly via the VAF (Mascó & Carrer, 1984). In contrast to the VMH, both the anterior and posterior LA inhibit sexual receptivity and these effects are mediated via the ST, but not via the VAF (Mascó & Carrer, 1980; Mascó & Carrer, 1984). All of these results demonstrate not only that the amygdala has the potential to influence sexual behavior in adult rats, but that the amygdala is one potential brain area that mediates the effects of social context on sexual behavior.

Rhesus Macaques

Evidence regarding the importance of the VMH for sexual behavior in rhesus macaques is limited. During mating, neuronal activity in the VMH increased in female rhesus macaques. Similarly, stimulation in the VMH resulted in female initiated solicits of the male not evidenced prior to stimulation (Oomura et al., 1988). Thus it appears that in rhesus macaques, the VMH is important for female initiated sexual behavior and this may be reflected as an increase in VMH neural activity during copulation.

Large bilateral lesions of the amygdala that spared only a portion of LA impaired some aspects of proceptive behavior, but not receptive sexual behavior in intact female rhesus macaques with functional ovaries (Spies et al., 1976). Sexual behavior was tested twice a day every other day between day 8 and 16 of the menstrual cycle both before and after lesion surgery, when estrogen levels are typically highest (Figure 1) (Spies et al., 1976). Behavioral tests consisted of a 10 minute pair-test. Female behaviors including being close to the male (proximity) and presentation of the female's hindquarters to the male (present) were used as indicators of female sexual initiation. Present after the male touched the female's waist (present to contact), mounts, intromissions, and ejaculations were scored as indicators of male sexual initiation and female sexual receptivity. Since the behaviors did not vary by cycle day, the data from all ten testing sessions were combined to compare behavior before and after lesion surgery. Female initiation of proximity is a proceptive behavior that increases markedly in concert with increases in preovulatory estradiol when monkeys are studied in multi-female groups with large testing areas (Wallen et al., 1984; Wallen, 1990). Under the more limited spatial conditions of the pair-tests used in this study, lesion-females initiated proximity to the

male less frequently following lesion surgery indicating decreased female motivation to approach the male. Lesion-females did not appear to be avoiding proximity to the male because they feared him as the rate of fear grimaces, a stereotypical fear response, to the male did not significantly increase following surgery. In contrast to proximity initiation, the rate of presents to the male did not differ between behavioral tests before and after surgery meaning the amygdala is not required for this female-initiated behavior (Spies et al., 1976). None of the measures of male-initiated behaviors or female sexual receptivity: Presents to contacts, mounting, intromission, and ejaculations, differed significantly after amygdala lesions in the females (Spies et al., 1976). What is particularly striking about these findings is that this same laboratory could not detect changes in female proximity initiation in relation to a female's hormonal state under these pair-test conditions (Johnson & Phoenix, 1976), yet they found a significant reduction in this behavior following lesions of the amygdala. This is strong evidence that the amygdala participates in regulating female sexual initiation. By contrast, lesion-female rhesus macaques continue showing receptive sexual behaviors meaning that the amygdala affects appetitive, but not consummatory female sexual behaviors. Although the females had intact, functional ovaries, the amygdala lesions did not alter measured levels of gonadal hormones (Spies et al., 1976) and therefore, any changes in sexual behavior resulting from amygdala lesions were not the result of changes in gonadal hormones levels.

The lack of effect of bilateral amygdala lesions on female sexual receptivity (Spies et al., 1976) may be a consequence of pair-testing methodology employed. Goy (1979) found that daily testing of pairs resulted in copulation on almost every day of the menstrual cycle. If given the opportunity, male rhesus macaques will mate with females

almost every day. This reflects that a female can physically mate at any time in her cycle (Wallen, 1990) but also the behavior more likely reflects male sexual motivation than it does female sexual motivation. Spies and colleagues (1976) tested amygdala lesion-females in pair-tests and found no difference in the number of mounts, intromissions, or ejaculations by the male. Male sexual behavior occurred daily in pair-tests indicating male sexual motivation is consistent (Goy, 1979). If only one female is present in the test situation, male sexual motivation may be high enough to preclude detectable changes in female sexual motivation to mate. Thus, the lack of a decrease in female receptive sexual behavior following amygdala lesions may reflect the social context of the sexual behavior testing. In groups with multiple females and one or more males, female initiated sexual behavior and copulation only occurs during the follicular phase of the menstrual cycle (Wilson et al., 1982; Wallen et al., 1984). Since there was a decrease in female initiated proximity to the male by lesion-females in pair-tests (Spies et al., 1976), it is likely that amygdala lesion-females tested in a large social group would show an even greater decrease in other proceptive behaviors, such as presents to the male. There would also be a reduction in male-initiated behaviors, such as mounts, intromissions, and ejaculations, but this might be because in large mixed-sex groups, females initiate almost all sexual interactions (Wallen, et al., 1984). Thus without female sexual initiation, little actual sexual activity will occur with lesion-females. Observing the sexual behavior of female rhesus macaques with neonatal bilateral amygdala lesions in a social group was one specific aim of this study in order to determine whether a decrease in sexual behavior is detected when females must compete for mating opportunities.

Hormonal Regulation in Adult Females

The hypothalamic-pituitary-gonadal (HPG) axis regulates gonadal hormone levels throughout the ovarian cycle in females. Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus and acts on the anterior pituitary causing the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH from the pituitary act on the gonad, the ovaries in females, which results in the production and release of progesterone and estrogen from the ovary (as reviewed by Goodman & Knobil, 1981). The HPG axis is maintained by a feedback loop in which estrogen acts on the medial preoptic area (mPOA) of the anterior hypothalamus, arcuate nucleus (ARC) of the medial basal hypothalamus, and secondarily at the level of the anterior pituitary in rats (Figure 3). In rats, the mPOA and its connections to the ARC are important for positive feedback of the HPG axis, whereas the ARC is important for the basal release of GnRH (Velasco & Taleisnik, 1971; Kawakami et al., 1973; Gallo & Osland, 1976). Although estrogen also acts on the pituitary in rats, this feedback does not alter LH and FSH increase, but rather increases the sensitivity of the pituitary to GnRH during positive feedback (Libertun, Orias, & McCann, 1974). In monkeys, estrogen acts primarily at the level of the anterior pituitary (Figure 4) to regulate LH positive feedback; however, the pulsatile release of GnRH is required for the proper functioning of the HPG axis feedback loop (Chongthammakun & Terasawa, 1993; Krey et al., 1975; Nakai et al., 1978; Plant et al., 1978; Xia et al., 1992).

Positive feedback occurs prior to ovulation when rising estrogen levels result in an increase in GnRH, LH, and FSH release in the rat (Figure 5) or increased LH and FSH release in primates (Figure 1). This increase in LH, known as the LH surge, is the trigger for ovulation in both rats and primates. Positive feedback occurs on the afternoon and

evening of proestrus in rats and during the peri-ovulatory phase in primates (Butcher et al., 1974; as reviewed by Knobil, 1974). In the rat, the period of positive feedback is often referred to as the critical period.

This section will focus on positive feedback during the four day rodent cycle and the follicular and peri-ovulatory phases of the primate ovulatory cycle to demonstrate what types of effects the amygdala has on ovarian cycles. The limited evidence in primates regarding amygdala influences on hormonal regulation pertains to the hormonal changes that occur during the follicular and peri-ovulatory phases and thus, hormonal changes that occur during the luteal phase will not be discussed.

Gonadal Hormone Regulation in Adult Female Rats

The medial preoptic area (mPOA) and the arcuate nucleus (ARC) are important for positive feedback and this section describes how these two hypothalamic regions are involved in positive feedback prior to discussing the influence of amygdala on gonadal hormone regulation. Influences of the amygdala on hormonal regulation have focused on the MeA, CoA, CeA, BlA, and LA, of which the MeA, CoA, and CeA all have direct connections to the mPOA and ARC (Figure 2) (as reviewed by Pitkänen, 2003).

Medial Preoptic Area (mPOA) and Arcuate Nucleus (ARC) of the Hypothalamus

The medial preoptic area (mPOA) of the hypothalamus is important for gonadal hormone regulation and facilitates positive feedback in rats. Serum and pituitary luteinizing hormone (LH) levels of intact rats increased after electrical stimulation in the mPOA in comparison to control animals receiving no stimulation (Kawakami et al., 1973; Kawakami & Higuchi, 1980). Thus, stimulation in the mPOA results in the

increased production and release of LH from the pituitary. Stimulation of the mPOA on any afternoon of the rat ovulatory cycle resulted in an increase in serum and pituitary LH (Kawakami et al., 1973) indicating this increase in LH following stimulation is not dependent on high estradiol levels. Though high estrogen levels are not required to produce an increase in LH following stimulation in the mPOA, the presence of some estrogen is required. In ovariectomized rats, ECS in the mPOA only produced an increase in LH in rats primed with estradiol benzoate (EB) and no increase was found in ovariectomized rats receiving no hormonal treatment (Kawakami & Higuchi, 1980). Stimulation in the mPOA can promote positive feedback and the LH surge in the presence of estradiol, but not in the absence of estradiol. Stimulation in the mPOA on any day of the ovulatory cycle produced an increase in LH, but the largest increase was observed on the afternoon of proestrus (Kawakami et al., 1973), which is when LH levels begin to increase as a result of rising estradiol levels and positive feedback in intact rats (Butcher et al., 1974). Thus, the presence of estrogen is required for an increase in LH as a result of stimulation in the mPOA and this increase in LH is largest when estradiol levels are also high.

While the mPOA is important for positive feedback, the efferent connections of the POA to the rest of the hypothalamus are also important for positive feedback. Transection of the efferent connections from the POA on the morning of proestrus prevented ovulation in female rats when measured the following morning (Velasco & Taleisnik, 1971). Ovulation still had not occurred four weeks following transection (Velasco & Taleisnik, 1971) demonstrating transection of the efferent connections of the mPOA had long-term effects on positive feedback. The mPOA has efferent connections

to the arcuate nucleus (ARC) of the hypothalamus (as reviewed by Pitkänen, 2003) and the ARC also facilitates positive feedback. Ovariectomized rats pretreated with EB showed an increase in serum LH following stimulation in the ARC (Gallo & Osland, 1976). Therefore, the projections from the mPOA are likely acting on the ARC to facilitate positive feedback when estrogen is present. The mPOA, ARC, and the connections between these two nuclei are involved in positive feedback and stimulation in the mPOA or ARC combined with increased estrogen levels facilitates positive feedback (Velasco & Taleisnik, 1971; Gallo & Osland, 1976).

Like the efferent connections of the POA, transection of the afferent connections to the POA on the morning of proestrus prevented ovulation when measured the following morning of estrus (Velasco & Taleisnik, 1971). However this effect did not persist and most of the rats had ovulated by four weeks following transection (Velasco & Taleisnik, 1971). Interrupting the input into the POA can interfere with positive feedback and prevent ovulation temporarily indicating at least some areas of the brain projecting to the POA have the potential to affect gonadal hormone regulation. The amygdala has direct connections to the mPOA and ARC (as reviewed by Pitkänen, 2003) and thus, may influence hormonal regulation by directly affecting the mPOA and/or the ARC.

Intact rats were used in the following studies on gonadal hormone regulation to demonstrate the influence of the amygdala on positive feedback and ovulation, whereas ovariectomized rats were used to demonstrate facilitatory influences of the amygdala. Ovulation was measured by presence of the ova in the oviducts and vaginal smears typically indicated the day of the cycle. All animals had at least two normal (4 or 5 day

cycles) cycles prior to amygdala manipulation to ensure that any changes in ovulatory cycles were a result of the manipulation.

Medial (MeA) and Cortical (CoA) Nuclei of the Amygdala

The mPOA and ARC facilitate positive feedback (Velasco & Taleisnik, 1971; Kawakami et al., 1973; Butcher et al., 1974; Gallo & Osland, 1976; Kawakami & Higuchi, 1980) and both the MeA and CoA project to the mPOA and ARC (Figure 2) (as reviewed by Pitkänen, 2003). Evidence from lesion studies supports a facilitatory influence of the MeA and shows bilateral electrolytic lesion of the MeA or both the MeA and the CoA inhibit positive feedback. In ovariectomized rats, positive feedback induced by a large injection of EB (7.0ug/100g BW) was prevented in animals with bilateral lesions of the MeA and CoA (Tyler & Gorski, 1980). While sham-operated females showed an increase in LH 53 hours after EB injection in comparison to baseline LH levels, females with MeA and CoA lesions had LH levels comparable to baseline values. Lesions of the MeA and CoA prevented EB induced positive feedback in ovariectomized rats (Tyler & Gorski, 1980) which suggests lesions of both the MeA and CoA would prevent positive feedback and ovulation in intact rats.

The evidence from the previous work indicates the MeA and/or the CoA facilitate positive feedback, but it does not elucidate whether both nuclei have a facilitating effect. Velasco and Taleisnik (1971) found bilateral lesions of the MeA on the morning of proestrus prevented ovulation in all animals when ovulation was measured the next morning on the day of estrus. These results demonstrate bilateral lesions of the MeA alone can inhibit positive feedback in ovariectomized and intact rats and indicate that the MeA has a facilitatory influence on gonadal hormones. There are no

lesion studies which examine the effect of the CoA alone on positive feedback or ovulation, however stimulation research, which will be discussed shortly, shows the CoA does not facilitate positive feedback and the inhibition of ovulation following combined lesions of the MeA and CoA is likely due solely to the facilitatory influence of the MeA (Beltramino & Telaisnik, 1978).

The effect of the MeA lesion preventing ovulation is likely not permanent. Bilateral electrolytic lesions of the MeA and CoA disrupted estrous cycles for 3-27 days following surgery and this disruption occurred more often for lesion animals than sham-operated controls. Females did resume cycling by three or four weeks following surgery (Tyler & Gorski, 1980; Chateau, Kauffmann, & Aron, 1984). When estrous cycles did resume, the length of cycles was longer in duration, typically five days, or alternated between four and five day cycles (Chateau et al., 1984). Since rats resumed estrous cycles several weeks following lesion of the MeA and CoA (Tyler & Gorski, 1980; Chateau et al., 1984), the effects of the lesion on positive feedback and ovulation are not permanent and these nuclei are not necessary for the long-term functioning of the HPG axis.

Stimulation in the MeA either in the morning or afternoon of proestrus had no effect on ovulation and all animals ovulated by the following morning (Ellendorff, Colombo, Blake, Whitmoyer, & Sawyer, 1973; Kawakami et al., 1973; Kawakami & Kimura, 1975; Carrillo, Rabii, Carrer, & Sawyer, 1977; Beltramino & Taleisnik, 1978). The only effect observed following stimulation in the MeA was an earlier increase in LH, but the timing of the LH surge did not differ (Beltramino & Taleisnik, 1978). Though these results do not demonstrate the facilitatory nature of the MeA, the results do show

that the MeA does not inhibit positive feedback. In order to demonstrate that the MeA facilitates positive feedback, stimulation should be done on a day of the ovulatory cycle when the LH surge is not expected to occur, for example diestrus II, to observe whether the stimulation can induce an LH surge and ovulation, but this experiment has not been done. However, stimulation in the MeA of EB primed ovariectomized females indicates the MeA can facilitate positive feedback following stimulation (Beltramino & Taleisnik, 1978). Ovariectomized females received an injection of EB three days before electrochemical stimulation in the MeA, which occurred two hours prior to the time of the LH surge observed in intact females. An LH increase and surge, which was comparable to the LH surge seen in intact controls on the afternoon of proestrus, occurred about three hours after electrochemical stimulation in the MeA. Non-stimulated EB primed ovariectomized females showed no change in LH levels. The increase in LH following stimulation in the MeA was dependent on the current strength and the amount of time the current was delivered in that 100μA for 60 seconds was required to produce an LH surge in ovariectomized females that was comparable to intact controls (Beltramino & Taleisnik, 1978).

In contrast to stimulation in the MeA, electrochemical stimulation in the CoA of ovariectomized EB primed rats did not increase LH levels and LH levels were similar to EB primed ovariectomized non-stimulated females (Beltramino & Taleisnik, 1978). Thus, the CoA does not facilitate positive feedback, but rather the CoA may have a weak inhibitory influence on positive feedback. Electrochemical stimulation in the CoA two hours prior to the LH surge resulted in two-thirds of females ovulating. All of the rats that ovulated had an LH surge comparable in size and timing to control animals. The

animals that did not ovulate did not have an LH surge (Beltramino & Taleisnik, 1978). It is possible the CoA has a weak inhibitory effect on positive feedback based on the location of the electrode within the CoA. In the females that did ovulate, the electrode tip was more dorsal and medially located in the CoA, whereas the electrode tip was more ventral and laterally located in females that did not ovulate (Beltramino & Taleisnik, 1978). Additional research considering the location of stimulation within the CoA is needed to understand if the CoA has an inhibitory effect on positive feedback.

In summary, the MeA has a facilitatory influence on positive feedback in rats, but this influence does not permanently affect the long-term functioning of the HPG axis. The CoA may have a weak inhibitory influence, but future stimulation and lesion research which examines the CoA alone is required to fully understand how the CoA may influence positive feedback.

Central Nucleus of the Amygdala (CeA)

The CeA does not influence positive feedback of the HPG axis despite the connections to both the mPOA and the ARC (as reviewed by Pitkänen, 2003). Electrochemical stimulation in the CeA had no effect on LH release in EB primed ovariectomized females and both stimulated females and control ovariectomized EB primed females showed low consistent levels of LH (Beltramino & Taleisnik, 1978). Stimulation in the CeA in ovariectomized females did not induce positive feedback as was shown following MeA stimulation (Beltramino & Taleisnik, 1978). Thus, stimulation in the CeA does not facilitate positive feedback.

In intact rats, stimulation in the CeA in the late morning or afternoon of proestrus had no effect on ovulation, with most animals ovulating by the morning of

estrus (Ellendorff et al., 1973; Beltramino & Taleisnik, 1978). Thus, the CeA did not inhibit ovulation or positive feedback. In summary, stimulation in the CeA did not facilitate or inhibit positive feedback and therefore, has no known influence on the HPG axis.

Basolateral (BlA) and Lateral (LA) Nuclei of the Amygdala

The BlA and LA have no direct projections to the mPOA or the ARC (Figure 2) (as reviewed by Pitkänen, 2003) and therefore any effect of the BlA and LA on positive feedback is likely mediated by another nucleus of the amygdala or another brain area. Despite normal ovulation following stimulation in the BlA and LA, delayed and blunted LH levels were observed when weak stimulation occurred just prior to the time of the LH surge (Ellendorff et al., 1973; Carrillo et al., 1977). However, weak stimulation in the BlA and LA two hours before the period of the LH surge had no effect on LH levels or the LH surge (Carrillo et al., 1977). Regardless of the timing of stimulation, ovulation is not affected by weak stimulation in the BlA and LA. However, when stronger electrical currents were used to stimulate the BlA or LA, an inhibitory effect on LH levels is observed. Electrochemical stimulation in either the BlA or the LA two hours before the critical period prevented ovulation and LH surges in all animals. A graded response was found for LH levels in that exposure to higher currents for longer time intervals (100µA for 30 seconds) resulted in greater decreases in LH levels and the prevention of ovulation (Beltramino & Taleisnik, 1978). If stimulation occurs just before the critical period rather than several hours before, a stronger current is required. When electrochemical stimulation in the LA occurred one half-hour before the critical period, at least 300µA was required to prevent ovulation. These conditions reduced LH on the evening of

proestrus, but only blocked ovulation in half of the animals (Kawakami & Kimura, 1975; Kawakami, Kimura, & Kawagoe, 1976). Therefore, the BlA and LA can inhibit positive feedback if stimulation occurs two hours prior to the critical period and a stronger current is used. If the stimulation occurs just before the critical period, an even larger current strength is required, but the effect may not inhibit ovulation in all animals.

Stria Terminalis

The amygdala projects to the hypothalamus via the stria terminalis (ST) and the ventral amygdalofugal pathway (VAF) (as reviewed by Pitkanen, 2003). Bilateral transection of the ST on the morning of proestrus prevented ovulation in all animals when measured on the following morning of estrus, whereas unilateral transection of the ST did not prevent ovulation (Velasco & Taleisnik, 1971). Therefore, the projections to the mPOA via the ST are important for positive feedback and ovulation. If the ST was bilaterally transected on the morning of proestrus and the mPOA was stimulated on the afternoon of proestrus, most of the animals ovulated by the following morning (Velasco & Taleisnik, 1971). The influence of the ST on the mPOA is stimulatory in nature and this stimulation in the mPOA is important for ovulation. In persistent estrus female rats, electrochemical stimulation in the MeA resulted in spontaneous ovulation in most animals, but if the ST was damaged during stimulation, none of the rats spontaneously ovulated (Velasco & Taleisnik, 1969). Therefore, the ST is important for the facilitatory influence of the MeA on positive feedback. The effects of ST damage are short-term effects and like damage to the MeA, damage to the ST only temporarily affects positive feedback and ovulation. Bilateral transection of the ST prevented ovulation for 10-15 days regardless of when the transection was done during the ovulatory cycle. After this

interruption of the cycle, females resumed normal ovulatory cycles (Velasco & Taleisnik, 1971; Döcke & Bao, 1978). In summary, the MeA projects to the mPOA via the ST and both the MeA and ST can facilitate ovulation and positive feedback. However, the projections from the MeA to the mPOA via the ST are not required for long-term functioning of positive feedback.

Summary

In female rats, positive feedback occurs following stimulation in the MeA (Kawakami et al., 1973; Kawakami & Kimura, 1975; Beltramino & Taleisnik, 1978), whereas lesions of the MeA can temporarily prevent positive feedback (Velasco & Taleisnik, 1971; Tyler & Gorski, 1980; Chateau et al., 1984). The CoA has a weak inhibitory effect on positive feedback following stimulation (Beltramino & Taleisnik, 1978), whereas the CeA has no influence on positive feedback (Ellendorff et al., 1973; Beltramino & Taleisnik, 1978). The BlA and LA have an indirect inhibitory influence on positive feedback following strong stimulation (Kawakami & Kimura, 1975; Kawakami et al., 1976; Beltramino & Taleisnik, 1978). Transection of the ST can prevent ovulation, but ovulation can occur following transection of the ST if the mPOA is stimulated (Velasco & Taleisnik, 1969). The MeA projects to the mPOA via the ST (as reviewed by Pitkänen, 2003) and both the MeA and ST can facilitate ovulation and positive feedback. Overall, the MeA has the largest influence on gonadal hormone regulation, but the CoA, BlA, and LA can have minimal effects depending on the context and these regions of the amygdala have the potential to integrate social information and directly influence gonadal hormone regulation.

Pubertal Onset in Female Rats

Pubertal onset in female rats is characterized by vaginal opening, or loss of the membrane covering the vagina, followed by first ovulation, which typically occurs around 37-40 days of age depending on the strain and laboratory (Bloch & Ganong, 1971; Relkin, 1971a, b; Döcke, 1974; Docke, Lemke, & Okrasa, 1976; Döcke, Rohde, Lange, & Dörner, 1980; Döcke, Rohde, & Dörner, 1983). From birth to puberty, rats experience two peaks of LH and FSH around 3 and 15 days of life (Figure 6) (Kamberi, deVellis, Bacleon, & Inglish, 1980). From birth to approximately four weeks of life, the hypothalamus is extremely sensitive to estradiol and the HPG axis is regulated by negative feedback. Hypothalamic desensitization to estradiol, which allows increases in LH and FSH from the pituitary resulting in increased follicle growth and estradiol levels, occurs around the fourth week of life culminating in vaginal opening and first ovulation around 40 days of age (as reviewed by Döcke, 1981).

Influence of the Amygdala on Pubertal Onset in Female Rats

Examination of the influence of the amygdala on pubertal onset in female rats has largely focused on the MeA. Bilateral electrolytic lesion studies were performed at different ages between birth and pubertal onset and this section is organized first by age of lesion in days and then by lesion of the anterior or posterior MeA, when applicable.

Bilateral electrolytic lesions of the amygdala in female rats at four or six days of age resulted in delayed vaginal opening by approximately 20 days in comparison to sham-operated controls (Relkin, 1971a,b). The lesion of the amygdala was not specific to one nucleus but rather included damage to the medial, cortical, central, basomedial, and basolateral nuclei and also damaged the beginning fibers of the stria terminalis. Body weight at age of vaginal opening was much greater in females with amygdala lesions in

comparison to sham-operated controls, but the females were also 20 days older at the time of vaginal opening. If differences in body weight were the only factor influencing this delay of vaginal opening, then body weight at the age of vaginal opening should be similar between females with amygdala lesions and control females. However, this greater body weight of lesion-females at the time of vaginal opening suggests the delayed effect is not due to body weight but rather an influence of the amygdala on the HPG axis and on vaginal opening (Relkin, 1971a,b). The delay in vaginal opening suggests the amygdala facilitates GnRH release via some unknown mechanism(s) at four days of age, when estradiol, LH, and FSH levels are declining (Kamberi et al., 1980). It is possible the amygdala tonically reduces the sensitivity of the hypothalamus to estradiol and removal of the amygdala at this time enhances the sensitivity of the hypothalamus to estradiol, thereby requiring less estradiol to maintain low GnRH levels. Additional research is needed to evaluate this hypothesis. Despite the delay in vaginal opening, normal follicular development occurred in the ovaries of the amygdala lesion-females after vaginal opening and uterine and ovarian weights were similar between lesion and sham-operated controls. Once vaginal opening occurred, the lesion of the amygdala had no effect on HPG axis or the reproductive tract of the females demonstrating the effect of the lesion specifically altered the timing of pubertal onset without having other lasting effects on reproduction (Relkin, 1971a,b).

Bilateral electrolytic lesions of the anterior MeA at 15 days of age resulted in delayed vaginal opening and first ovulation by approximately four days in comparison to sham-operated controls (Döcke et al., 1980). These results are similar to the effect seen when lesion of the entire amygdala occurred at four or six days of age however, the delay

in vaginal opening is only four days in comparison to the 20 day delay seen following lesions at four or six days of age (Relkin, 1971a,b; Döcke, et al., 1980). Ovariectomy at 14 days of age resulted in an increase in LH, measured at 18 days of age, in sham-operated controls which demonstrates at this age, the HPG axis is regulated by negative feedback from estradiol. However, females ovariectomized at 14 days of age that received bilateral lesions of the anterior MeA at 15 days of age did not show this elevation in LH following ovariectomy and had LH levels comparable to control females of the same age with intact gonads and no amygdala lesions. Thus, lesions of the anterior MeA at 15 days of age increase estradiol sensitivity of the hypothalamus and prevent the LH rise following ovariectomy, possibly because non-gonadal sources of estrogen are sufficient to maintain negative feedback. If lesions of the anterior MeA at 15 days of age increase estradiol negative feedback sensitivity, then low levels of estradiol should maintain low GnRH levels, preventing or dampening the increases of LH and FSH that are typically observed at this age (Kamberi et al., 1980). Failure to have the normal rise in LH and FSH at 15 days of age may explain the delay in vaginal opening observed in these animals (Döcke et al., 1980). Thus, at 15 days of age, the anterior MeA appears to facilitate GnRH release by decreasing the sensitivity of estradiol negative feedback.

At 21 or 22 days of age, bilateral electrolytic lesion of the anterior MeA resulted in earlier vaginal opening and first ovulation by about four to six days in lesion-females in comparison to sham-operated controls (Döcke, 1974; Docke et al., 1976; Döcke et al., 1980), which suggests at this age the anterior MeA has an inhibitory influence. If females were ovariectomized at 20 days of age and then received sham or bilateral lesions of the anterior MeA at 21 days of age, sham-operated controls showed an increase

in LH on day 25 indicating the HPG axis was escaping from negative feedback. Lesion of the anterior MeA at 21 days of age also resulted in an increase in LH following ovariectomy, however lesion-females showed a significantly greater increase in LH in comparison to sham-operated controls (Döcke et al., 1980). There is no reason to expect that the pituitary differs between the sham and lesion-females, thus it is likely that lesion-females are releasing more GnRH from the hypothalamus resulting in the greater increase in LH from the pituitary. In addition, daily injections of estradiol benzoate (EB) given the day after ovariectomy, 21 days of age, for four days resulted in suppressed LH levels in both sham-operated and lesion-females (Döcke et al., 1980). Therefore, lesions of the anterior MeA at 21 days of age are not influencing the estradiol sensitivity of negative feedback but rather appear to be increasing GnRH release from the hypothalamus. When FSH levels were measured daily from blood pooled from eight to ten animals, females receiving bilateral lesions of the anterior MeA at 21 days of age showed an increase in FSH on 22-24 days of age that was not found in sham-operated controls (Döcke et al., 1983). This increase in FSH could result in earlier development of the follicle, which would explain why lesion of the anterior MeA at this age results in earlier pubertal onset and it would also result from an increase in GnRH release. However, LH levels did not differ between lesion and sham-operated controls (Döcke et al., 1983), which supports the finding that MeA lesions do not influence negative feedback at 21 days of age (Döcke et al., 1980). If lesions of the anterior MeA increase GnRH release and ultimately FSH release, then this would support an inhibitory influence of the anterior MeA at 21 days of age. Sometime between 15 and 21 days of age, the influence of the anterior MeA on pubertal onset appears to shift in that lesions of the anterior MeA at 15 days of age or

earlier delay pubertal onset whereas lesions of the anterior MeA at 21 days of age result in earlier pubertal onset (Relkin, 1971a, b; Döcke, 1974; Docke et al., 1976; Döcke et al., 1980). It is currently not clear why lesions of the anterior MeA at different age points result in different effects on pubertal onset. Lastly, in the 16 days following vaginal opening, all animals had two to three ovulatory cycles regardless of treatment and the first estrus cycle was approximately six days after vaginal opening (Döcke, 1974; Docke et al., 1976). Although lesions of the anterior MeA result in earlier pubertal onset at 21 days of age, the lesions do not have long term affects on hormones or ovarian cycles after pubertal onset, which suggests the mechanisms regulating pubertal onset are different than the mechanisms regulating adult ovarian cycles.

In contrast lesions of the anterior MeA, lesions of the posterior MeA at 21 days of age had no effect on age at vaginal opening and first ovulation and both lesion-females and sham-operated controls had vaginal opening and first ovulation around 40 days (Döcke, 1974; Döcke et al., 1976). There were also no lasting effects of this lesion after puberty as the first estrus cycle occurred approximately six days after vaginal opening for both lesion and sham-operated controls (Döcke et al., 1976). In addition, lesion-females and sham-operated controls both had two to three ovulatory cycles in the 16 days after vaginal opening (Döcke, 1974). Thus, the posterior MeA has no effect on pubertal onset and the facilitatory nature of the MeA is limited to the anterior MeA.

Bloch and Ganong (1971) found no effect on age at vaginal opening or first ovulation in females after bilateral electrolytic lesions of the MeA at 21 days of age, but the beginning of the stria terminalis originating in the amygdala was also damaged. It is not clear whether the lesion involved the anterior or posterior MeA, so it is possible the

lesion was of the posterior MeA and supports the data from Döcke and colleagues (1974, 1976). It is also possible that damaging the stria terminalis prevents the effect of the MeA lesion on influencing the timing of pubertal onset. Thus, it is not yet clear how the stria terminalis is involved in mediating the effects of the anterior MeA on the ARC.

Age at vaginal opening and first ovulation did not differ between lesion-females and sham-operated controls when bilateral electrolytic lesions of the anterior MeA occurred at 26 days of age (Döcke et al., 1976; Döcke et al., 1980). When females were ovariectomized the day before lesion of the anterior MeA or sham surgery, 25 days of age, LH levels increased in both lesion and sham-operated females by 29 days of age indicating the HPG axis was escaping from negative feedback in both lesion and sham-operated controls. Lesions of the anterior MeA at 26 days of age do not influence pubertal onset or interfere with negative feedback of the HPG axis (Döcke et al., 1976; Döcke et al., 1980).

Though lesions of the anterior MeA at 26 days of age had no effect on pubertal timing, lesions of the posterior MeA resulted in delayed first ovulation by about three days, while age at vaginal opening did not differ from sham-operated controls (Döcke et al., 1976). The delay in first ovulation is likely a result of the lesion of the posterior MeA disrupting positive feedback or the development of positive feedback, since the positive feedback mechanism is developed at approximately four weeks of age (as reviewed by Döcke, 1981).

Electrical stimulation at 26 days of age shows the MeA may facilitate gonadotropin production and/or release (Kawakami & Terasawa, 1972). Electrical stimulation of the MeA for 30 minutes at approximately 26-29 days of age resulted in an

increase in pituitary LH immediately after stimulation in MeA lesion animals in comparison to sham-operated controls. Thus, the stimulation resulted in an increase in the production of LH. Sham-operated controls had bipolar electrodes implanted in the MeA but received no stimulation and these animals did not have hormone levels that differed from unmanipulated control females. Thus, the implanting of the electrodes was not responsible for the increase in pituitary LH observed after stimulation of the MeA. Despite the increase in pituitary LH immediately following stimulation in the MeA, there was no increase in serum levels of LH demonstrating the increased production of LH in the pituitary did not result in an increased release of LH. Pituitary FSH or serum FSH levels did not differ between stimulated and sham controls. When hormone levels were measured 30 minutes after the cessation of stimulation, pituitary levels of LH and FSH as well as serum FSH levels were greater in females receiving MeA stimulation in comparison to sham controls. The effect of the electrical stimulation in the MeA continued to occur after the stimulation resulting in an increase in the production of LH and FSH as well as the release of FSH, when only an increase in the production of LH was observed immediately after stimulation. Increased production of FSH and LH and increased release of FSH were also found when stimulation occurred for 30 minutes a day for three consecutive days beginning at 26 days of age demonstrating that short periods of daily stimulation do not alter the effects of MeA stimulation (Kawakami & Terasawa, 1972). Unfortunately, it is not clear whether the electrical stimulation occurred in the anterior or posterior MeA, the data do support the data showing the posterior MeA has a facilitatory influence on positive feedback at 26 days of age (Döcke et al., 1976).

At 32 days of age, lesions of either the anterior or posterior MeA resulted in delayed vaginal opening and first ovulation by approximately four or five days in comparison to sham-operated controls (Döcke et al., 1976). Thus, both the anterior and posterior MeA facilitate positive feedback at 32 days of age and disruption of these areas delays pubertal onset.

In summary, at four or six days of age some nucleus (nuclei) of the amygdala facilitates GnRH release, possibly by influencing the sensitivity of estradiol negative feedback and this influence continues until at least 15 days when the anterior MeA still demonstrates this effect. Between 15 and 21 days of age, something changes and the anterior MeA appears to inhibit GnRH release by 21 days of age, yet the reason for these specific age effects are not known. This inhibiting effect of the anterior MeA at 21 days of age is brief and disappears by 26 days of age as lesions of the anterior MeA at 26 days of age had no effect on pubertal onset. However, by 32 days of age, the anterior MeA facilitates positive feedback. The posterior MeA has no effect on pubertal onset at 21 days but at both 26 and 32 days, the posterior MeA facilitates positive feedback and disruption of this nuclei delays pubertal onset. The MeA has an influence on the timing of pubertal onset, but age and location within the MeA must be considered to understand the nature of these effects.

Adult Female Rhesus Macaques

Unlike rats, the feedback loop in rhesus macaques has estrogens feeding back primarily on the pituitary rather than the hypothalamus (Figure 4). The ARC in the medial basal hypothalamus (MBH) regulates GnRH release in rhesus macaques, but positive feedback of the HPG axis is primarily regulated by the pituitary and the ovary

(as reviewed by Knobil, 1974). Although the ARC is not directly involved in the process of positive feedback, GnRH release from the ARC is required for positive feedback to occur. Bilateral lesions of the MBH including the ARC reduced LH levels within 72 hours after the lesion and levels did not increase prior to the end of the study, three weeks following the lesion (Plant et al., 1978). Decreased LH levels following lesions of the ARC are thought to be a consequence of decreased GnRH release. If GnRH was administered to monkeys with MBH lesions that included the ARC, animals responded with an LH surge, supporting the hypothesis that decreased LH levels following lesions of the ARC are the result of decrease GnRH release (Plant et al., 1978). Replacement of pulsatile GnRH was sufficient to induce an LH surge, but rapid changes in GnRH as a result of estradiol feedback do not appear to be required for the LH surge. Lesions of the MBH that did not affect the ARC produced no alterations in LH indicating the ARC is the area in the MBH important for basal release of GnRH (Plant et al., 1978).

The mPOA is important for gonadal hormone regulation in rats (Velasco & Taleisnik, 1971; Kawakami et al., 1973; Kawakami & Higuchi, 1980), but input into the ARC from the mPOA is not required for hormonal regulation in monkeys. Deafferentation of the MBH in rhesus macaques produced no change in basal LH levels, nor did it have an effect on the LH surge following estradiol administration (Krey et al., 1975). Thus, afferent projections to the MBH are not required for the basal release of GnRH.

In rhesus macaques, the amygdala projects to the hypothalamus via the ST and VAF (as reviewed by Amaral et al., 1992), but it is not known whether the amygdala projects directly to the ARC. Lesions of the amygdala in individually housed rhesus

macaques did not influence positive feedback of the HPG axis (Spies et al., 1976). In intact rhesus macaques, bilateral lesions of the amygdala were done using a heated metal loop. Since the lesion device did not target specific nuclei of the amygdala, the areas of amygdala damage varied between animals. In the animals with the most damage, all of the amygdala but an area of the LA was destroyed. The female with the least amygdala damage had more damage in the caudal amygdala surrounding the MeA, CoA and CeA. This female also had some damage to the optic tract and internal capsule (Spies et al., 1976). Following lesion surgery, three of the five animals had a peri-ovulatory rise in estradiol and LH and rise in P during luteal phase, indicative of ovulation. The other two monkeys did not have an LH surge during the sixty days after surgery, but they also did not have an LH surge in the cycle prior to surgery. Thus, it is difficult to say that the lack of the LH surge after surgery is an effect of the surgery. All of the females that showed an LH surge following surgery also showed an LH surge in response to implants of silastic EB capsules on the third day after menses. One animal that did not exhibit an LH surge during the sixty days after surgery had an increased LH response after EB implants. Endogenous estradiol levels were too low to produce an LH surge, but when given exogenous estradiol this female was capable of showing an LH surge. Thus, positive feedback was not affected in this female despite her lack of ovulatory cycles following surgery. The second female that did not show normal cycles following surgery still did not release LH in response to EB implants. Interestingly, this female had the smallest amount of damage to the amygdala but had damage to the internal capsule and optic tract (Spies et al., 1976). Thus, it is possible her failure to exhibit an LH surge was due to

damage sustained outside the amygdala. In summary, bilateral lesions of the amygdala did not influence positive feedback in female rhesus macaques.

The same female rhesus macaques discussed in the previous paragraph were ovariectomized several months after lesion surgery. When implanted with silastic EB capsules three weeks after ovariectomy, all of the females showed the expected increase in LH (Spies et al., 1976). Positive feedback of the HPG axis in ovariectomized monkeys, like in intact monkeys, was not affected by the amygdala lesion.

In contrast to Spies and colleagues, Erikson and Wada (1970) found bilateral ablation of the amygdala increased the length of ovulatory cycles in most female rhesus macaques. All of the females used in this study showed normal ovulatory cycles prior to surgery, which were 17-31 days in length. Cycles were measured daily by vaginal sloughing patterns and by checking for menses in cage pans. Only one of the five animals showed normal cycle duration following bilateral ablation of the amygdala. Three of the five animals showed cycles lengths more than 200 days following surgery however, this increased cycle duration didn't occur until the second cycle after ablation. If the increased cycle duration was a result of amygdala ablation, then it would likely occur immediately after removal of the amygdala rather than during the second cycle after ablation. Since there were no control animals in this study, it is not clear whether the increased cycle duration was a result of amygdala ablation or another factor. Despite the increased cycle duration, all females had resumed normal cycles by one year following surgery (Erikson & Wada, 1970). If there is an inhibition of positive feedback following amygdala ablation, then the effect is not permanent as was demonstrated in rats.

The evidence regarding how the amygdala influences hormonal regulation in rhesus macaques is conflicting. Bilateral lesions of the amygdala had no effect on positive feedback or hormonal profiles, whereas bilateral ablation of the amygdala increased ovulatory cycle length. Bilateral ablation of the amygdala may cause more damage to the surrounding areas as well as the stria terminalis, which may have resulted in increased cycle length. Since there were no hormonal measures collected by Erikson and Wada (1970), it is difficult to state whether the irregular cycles were due to low levels of gonadal hormones preventing an LH surge or whether bilateral ablation affected LH response to estrogen and positive feedback. Since the ST was largely intact following bilateral lesions of the amygdala and hormone levels did not change, the data indicate the amygdala does not influence gonadal hormone regulation in adult individually housed rhesus macaques.

Deafferentation or lesions of the MBH and ARC decrease GnRH release causing a decrease in LH release from the pituitary (Krey et al., 1975). Since positive feedback of the HPG axis occurs at the level of the anterior pituitary in monkeys, the amygdala would have to influence GnRH release in order to alter LH and estradiol levels. Lesions of the amygdala might have had a small influence on GnRH release that was not apparent when viewing LH or estradiol levels. It is also possible the amygdala has no direct projection to the ARC and therefore, would not alter gonadal hormones. One way to determine whether the amygdala influences the ARC and alters GnRH levels in rhesus macaques is to examine the effect of the amygdala on pubertal onset. If the amygdala influences GnRH levels, then pubertal onset should be altered in animals with lesions of the amygdala.

Pubertal Onset in Female Rhesus Macaques

Unlike in rats, in rhesus macaques the HPG axis is inactive from birth until just before puberty and pubertal onset is initiated by the release of GnRH from the hypothalamus (as reviewed by Plant, 2001). Briefly, the median eminence of the hypothalamus releases gonadotropin releasing hormone (GnRH) in a pulsatile fashion, which acts on the anterior pituitary gland, resulting in the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Increasing levels of LH and FSH promote the release of estrogen and testosterone from the gonads (Figure 4) (as reviewed by Plant, 2001). These hormones are then regulated by the positive or negative feedback mechanisms found in adult rhesus macaques, as previously described (see Knobil, 1974 for review). Menarche and first ovulation are measures of pubertal onset in rhesus macaques and both indicate GnRH is being released from the ARC (Norman & Spies, 1981). The mechanisms regulating pubertal onset in rhesus macaques differ from those in rats in two ways: in rhesus macaques, estradiol feeds back on the pituitary rather than the hypothalamus and the HPG axis is quiescent in juvenile rhesus macaques, unlike in rats where negative feedback maintains low levels of hormones (as reviewed by Döcke, 1981; as reviewed by Plant, 2001). Looking at the timing of pubertal onset following lesions of the amygdala in rhesus macaques will highlight whether the amygdala is capable of influencing GnRH release from the hypothalamus at a time when hormone levels are typically low.

Influence of the Amygdala on Pubertal Onset in Female Rhesus Macaques

Lesions of the amygdala, largely the LA and BlA, at 10-13 months of age had no effect on the age at first menarche (mean age 29 months) and first ovulation (mean age 45

months) in comparison to unmanipulated control females (Norman & Spies, 1981). Three of the six animals had damage to the MeA and though age at menarche did not differ, age at first ovulation was delayed for one animal (56 months) and another animal never ovulated in comparison to the control females experiencing first ovulation around 43 months of age. Thus, damage to the MeA at 10-13 months of age resulted in a delay of first ovulation in two out of three animals, which suggests a disruption of positive feedback in these animals. However, there was no overall significant effect in age at menarche or first ovulation between lesion and control animals (Norman & Spies, 1981).

Knowing that age was important when considering the effects of the MeA on pubertal onset in rats, it is important to consider the age at which the lesion occurred. In rhesus macaques, maturation of the amygdala occurs around eight months of age (Payne, Machado, Bliwise, & Bachevalier, 2010), meaning the lesions of the amygdala in this study (10-13 months) occurred after the amygdala was developed (Norman & Spies, 1981). In rhesus macaques, the greatest developmental increase in volume of the amygdala occurred during the first month (Payne et al., 2010) and therefore, lesions of the amygdala around this time may be the most promising time to find an effect on pubertal onset.

With regards to age at menarche and first ovulation, there were no differences between lesion and control animals, but there was also little variation in either group (Norman & Spies, 1981). The lack of variation in pubertal onset in these females may be a result of indoor individual housing beginning at 18 months of age. In rhesus macaques housed outdoors, around 20-40% of females experience first ovulation at 30 months of age, whereas the remaining females experience first ovulation at 42 months of age

(Wilson, Gordon, Blank, & Collins, 1984). Social rank influences first ovulation in rhesus macaques living in large, social groups in that lower-ranking animals tend to reach first ovulation later, at 42 months of age (Zehr, et al., 2005). Both the control and amygdalectomized females reached menarche at approximately 30 months of age, but first ovulation did not occur until 42 months of age, which is when lower-ranking females living in a social group tend to reach ovulation (Norman & Spies, 1981; Zehr et al., 2005). Thus, the effects of amygdala lesions on pubertal onset might differ if pubertal onset were measured in females living in large, social groups subject to the rules of the social hierarchy.

In support of the hypothesis that influences of the amygdala may differ between group and individually housed animals, a study in mice found differences in ovarian and uterine weights between lesion and sham-operated animals was only observed in group housed mice (Pasley, Powell, Cernosek, & Cernosek, 1978). Female mice received bilateral lesions of the amygdala at 30 days of age and then housed either individually or in groups of eight to ten animals. There were no differenced in ovarian or uterine weights between individually housed intact, sham-operated, or amygdala lesion-females at 60 days of age. Amygdalectomized females living in groups had similar ovarian and uterine weights to individually housed animals, whereas intact and sham-operated females living in groups had lower ovarian and uterine weights (Pasley et al., 1978). Thus, intact and sham-operated females living in groups likely had suppressed ovarian function as a consequence of living in a group, the Lee-Boot effect (1955, 1956). In this case, the effect of the amygdala on the HPG axis and ovarian functioning was only observed in animals living in groups (Pasley et al., 1978) and therefore, the lack of an

effect of the amygdala on pubertal onset found by Norman and Spies (1981) may be a result of using individually housed animals. There is limited evidence available in rhesus macaques regarding the influence of the amygdala on pubertal onset and the results from Norman and Spies may be affected by the time of the lesion, using individually housed animals, and the lack of variation found in menarche and first ovulation.

Specific Aims and Hypotheses

The current study examined the influence of neonatal bilateral excitotoxic lesions of the amygdala on sexual behavior and pubertal onset in rhesus macaque females living in large, complex social groups. The use of neonatal excitotoxic amygdala lesions, which protect fibers of passage, while the amygdala is rapidly developing in combination with the use of females living in social groups will aid in our understanding of how or if the amygdala influences sexual behavior and pubertal onset in female rhesus macaques.

Specific Aim #1: Examine the effects of neonatal amygdala lesions on menarche and first ovulation in female rhesus macaques living in large, social groups.

Hypothesis 1: Amygdala lesioned female rhesus macaques will experience earlier menarche, if social rank predicts age at menarche, and first ovulation because social cues, such as social rank, cannot influence the timing of pubertal onset in lesion-females.

Hypothesis 2: Lesions of the amygdala will eliminate the variation in pubertal timing typically observed in females living in social groups because social rank cannot influence the variation in timing of pubertal onset.

Specific Aim #2: Examine the effects of neonatal amygdala lesions on the sexual behavior of pubertal female rhesus macaques living in large, social groups.

Hypothesis 3: Female rhesus macaques with bilateral lesions of the amygdala will show decreased sexual behavior in social groups because they must compete with other females for mating opportunities with the male(s).

*Note: References for the general introduction were combined with references for the general discussion and are located at the end of the discussion.

Figure Captions

Figure 1. Plasma estradiol, progesterone, LH, and FSH concentrations in rhesus macaques relative to the LH peak.

Figure 2. Connections between nuclei of the amygdala and areas of the hypothalamus influencing gonadal hormone regulation. Solid lines indicate moderate to heavy projections. Dashed lines indicate light projections.

Figure 3. Positive feedback loop of the HPG axis in rats with estrogen acting primarily on the preoptic area (POA) of the anterior hypothalamus (AH) and secondarily at the anterior pituitary.

Figure 4. Feedback loop of the HPG axis in primates regulating both positive and negative feedback, with feedback occurring primarily at the anterior pituitary and secondarily at the arcuate nucleus (ARC) of the medial-basal hypothalamus.

Figure 5. Plasma estradiol, progesterone, LH, and FSH levels in the rat during the four day estrous cycle.

Figure 6. Serum GnRH (a), FSH (b), LH (c), Estradiol (d), and Progesterone (e) levels from birth to 35 days after birth, just before vaginal opening in female rats (modified from Kamberi et al., 1980).

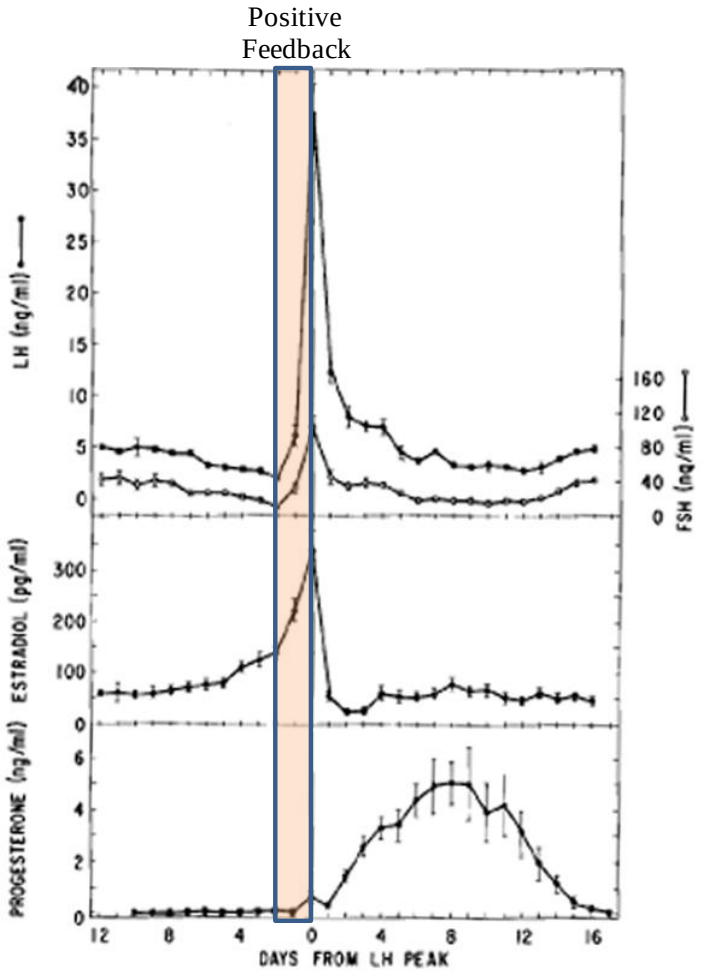

Positive
Feedback
LH (ng/ml)
FSH (ng/ml)
ESTRADIOL (pg/ml)
PROGESTERONE (ng/ml)
DAYS FROM LH PEAK

Figure 1.

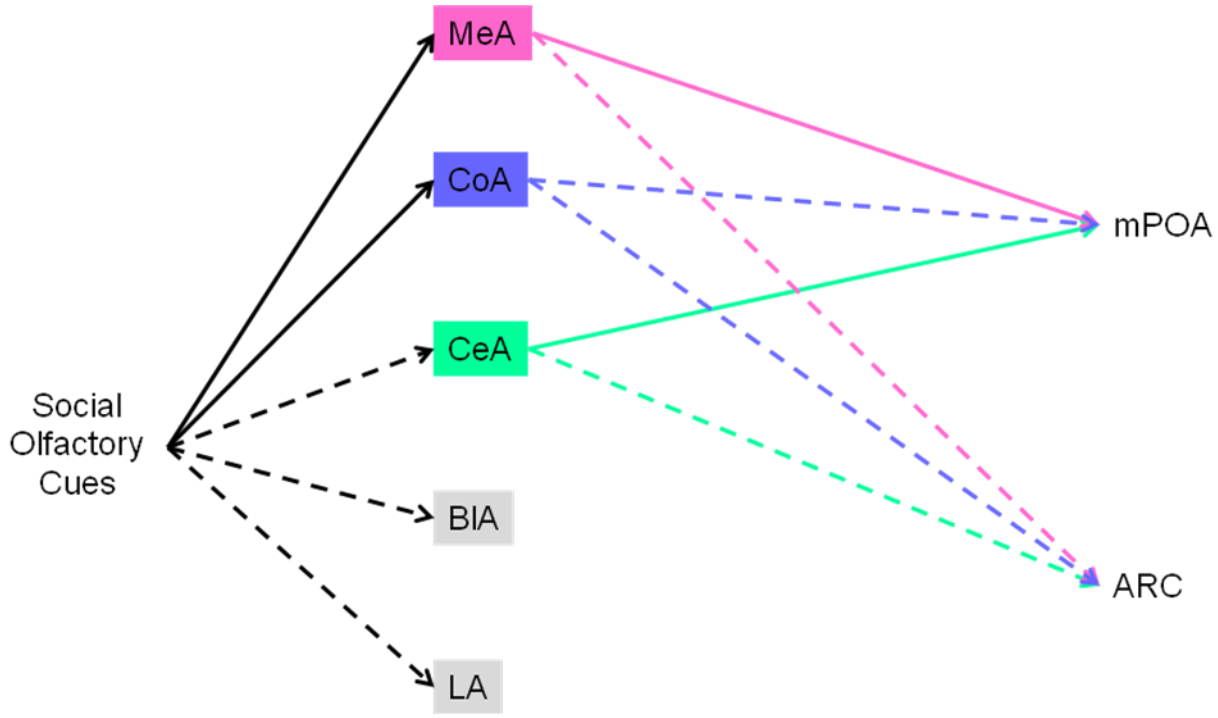


Figure 2.

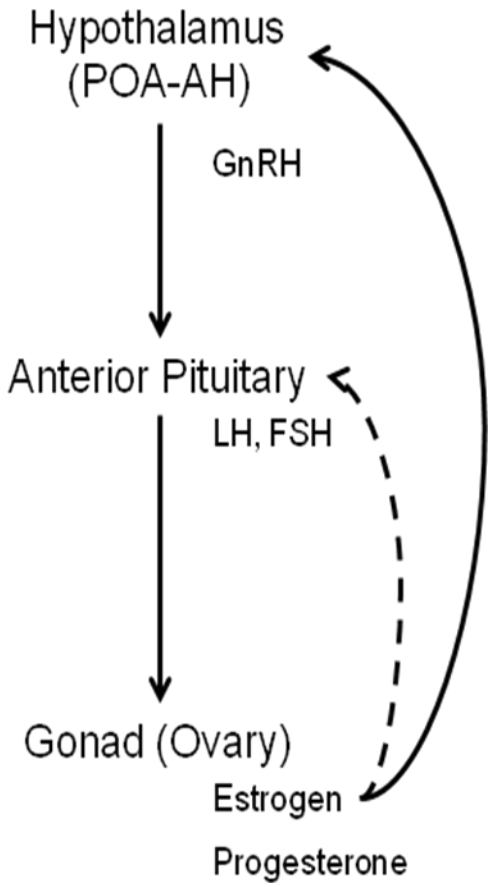

Hypothalamus
(POA-AH)
GnRH
Anterior Pituitary
LH, FSH
Gonad (Ovary)
Estrogen
Progesterone

Figure 3.

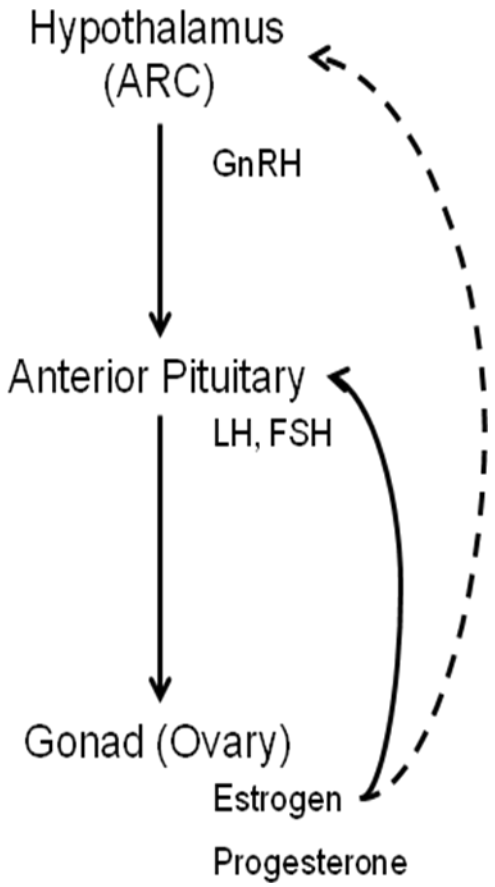

Hypothalamus
(ARC)
GnRH
Anterior Pituitary
LH, FSH
Gonad (Ovary)
Estrogen
Progesterone

Figure 4.

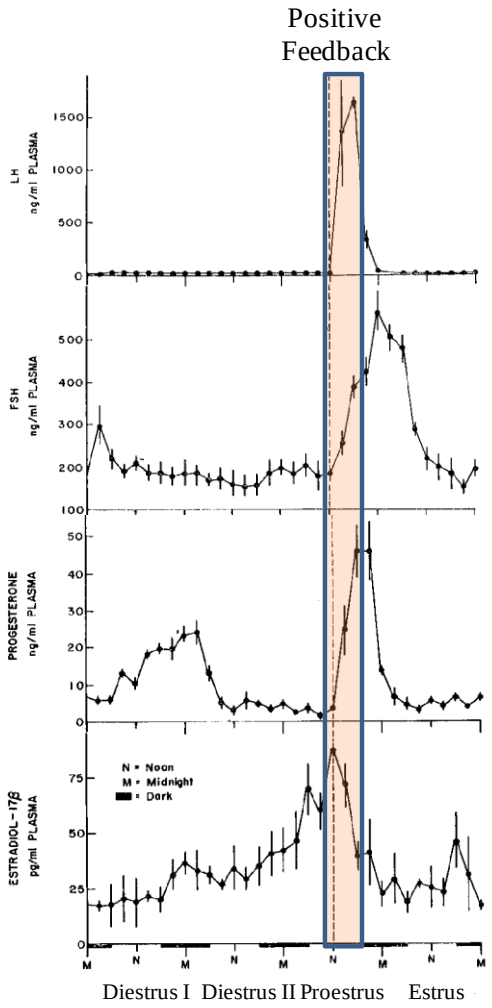
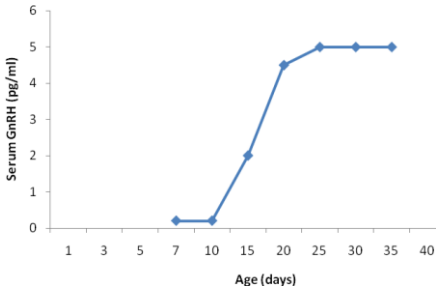
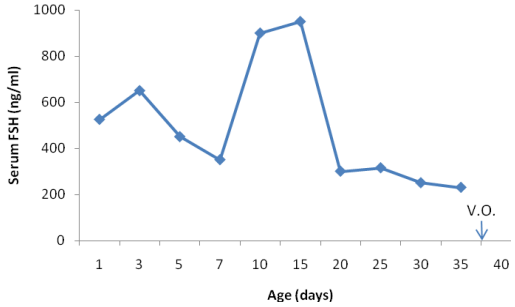

Positive
Feedback
LH
ng/ml PLASMA
1500
1000
500
0
FSH
ng/ml PLASMA
500
400
300
200
100
PROGESTERONE
ng/ml PLASMA
50
40
30
20
10
0
ESTRADIOL-17β
pg/ml PLASMA
75
50
25
0
N = Noon
M = Midnight
= Dark
M N M N M N M N M
Diestrus I
Diestrus II
Proestrus
Estrus

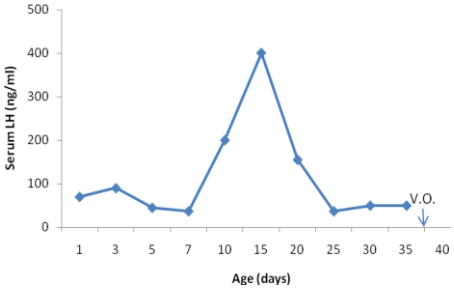
Figure 5.



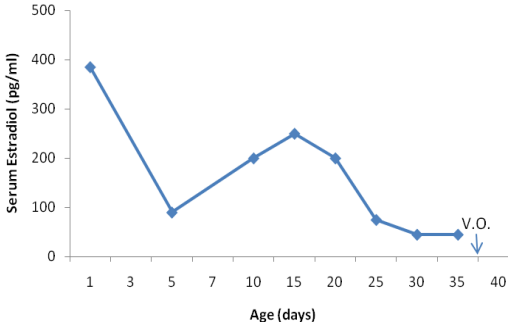
a.



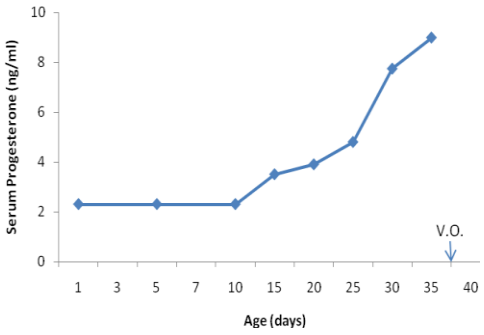
b.



c.



d.



e.

Figure 6.

Running head: Primate Puberty

Environmental and social influences on neuroendocrine puberty and behavior in macaques and other nonhuman primates

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**This manuscript discusses both males and females, but the research in this dissertation is limited to females.

Abstract

"Puberty is the developmental period when the hypothalamic-pituitary-gonadal (HPG) axis is activated, following a juvenile quiescent period, and reproductive capacity matures. Although pubertal events occur in a consistent sequence, there is considerable variation between individuals in the onset and timing of pubertal events, with puberty onset occurring earlier in girls than in boys. Evidence in humans demonstrates that social and environmental context influences the timing of puberty onset and may account for some of the observed variation. This review analyzes the nonhuman primate literature, focusing primarily on rhesus macaques (*Macaca mulatta*), to examine the social and environmental influences on puberty onset, how these factors influence puberty in males and females, and to review the relationship between puberty onset of adult neuroendocrine function and sexual behavior. Social and environmental factors influence the timing of puberty onset and pubertal events in nonhuman primates, as in humans, and the influences of these factors differ for males and females. In nonhuman primates, gonadal hormones are not required for sexual behavior, but modulate the frequency of occurrence of behavior, with social context influencing the relationship between gonadal hormones and sexual behavior. Thus, the onset of sexual behavior is independent of neuroendocrine changes at puberty; however, there are distinct behavioral changes that occur at puberty, which are modulated by social context. Puberty is possibly the developmental period when hormonal modulation of sexual behavior is organized, and thus, when social context interacts with hormonal state to strongly influence the expression of sexual behavior."

Keywords: Puberty onset, Social influence, Environmental influence, Sex differences, Nonhuman primate, Sexual behavior

"Puberty is the transitional period between juvenile and adult life stages, resulting in mature reproductive function. Although the stages of pubertal development are relatively consistent, there is considerable variation in the timing of puberty between individuals (Marshall & Tanner, 1969, 1970). Such variation in girls has been suggested to result from physiological factors such as body weight, as well as social factors such as parental divorce, father absence, or the presence of a stepfather, indicating that environmental influences may account for some of the variation in puberty onset (Graber et al., 1995; Moffit et al., 1992; Rowe, 2000; Tither & Ellis, 2008; Wierson et al., 1993). There are sex differences in the influence that environmental factors have on the timing of puberty; for example, body weight and stepfather presence influenced the timing of puberty onset in girls, but had no effect in boys, and parental divorce resulted in earlier puberty in girls, whereas it delayed puberty in boys (Belsky et al., 2007; Bogaert, 2005; Graber et al., 1995; Rowe, 2000; Semiz et al., 2009; Wierson et al., 1993). This review discusses environmental factors that alter the timing of puberty and produce variation in the timing and tempo of pubertal change as well as sex differences in the effect of these factors in nonhuman primates. In order to provide a better understanding of the sex difference(s) in environmental regulation of puberty timing, the effects of environmental factors on puberty onset and reproductive maturation are described separately for males and females in this review. Environmental factors likely alter the timing of puberty by influencing the neurobiological factors regulating puberty onset and pace, which reflect alterations in gonadotropin-releasing hormone (GnRH) release; however, the neurobiological factors regulating GnRH release itself are an active area of investigation

and discussion of these factors is beyond the scope of this review (see Plant, 2001 for a review).

Puberty Onset

Puberty is characterized by increased activation of the hypothalamic-pituitary-gonadal (HPG) axis as a result of increased gonadotropin-releasing hormone (GnRH) release (Suter et al., 1998; Watanabe & Terasawa, 1989). In primates, GnRH released from the arcuate nucleus of the medial basal hypothalamus acts on the anterior pituitary, resulting in the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH then act on the gonad, resulting in the synthesis and release of inhibin as well as steroid hormones such as estradiol (E2), progesterone, and testosterone (T) (Grumbach & Styne, 2003; Knobil, 1974, 1980). Inhibin released from the gonad acts on the anterior pituitary via a negative feedback loop inhibiting FSH, but not LH, release in both males and females (Figure 1; Molskness et al., 1996; Ramaswamy et al., 2000; Ramaswamy & Plant, 2001). In males, T release regulates GnRH release through a negative feedback loop (Figure 1a; Plant & Dubey, 1984). In contrast to males, in females, E2 negative and positive feedback occurs primarily at the level of the anterior pituitary and secondarily at the level of the hypothalamus (Figure 1b; Chongthammakun & Terasawa, 1993; Nakai et al., 1978; Plant et al., 1978; Xia et al., 1992). In adult female rhesus macaques, lesions of the arcuate nucleus decreased LH resulting in a cessation of ovulatory cycles, which were restored with administration of pulsatile GnRH (Plant et al., 1978). The arcuate nucleus, while required for pulsatile GnRH release, is not required for E2 feedback of the HPG axis as consistent pulsatile GnRH was sufficient to maintain ovarian cycles. No matter the site of feedback, pulsatile GnRH is required

for normal ovulatory cycles and testicular function (Plant & Dubey, 1984; Plant et al., 1978).

In women and female rhesus macaques, there is an increase in progesterone concurrent with the LH surge, just prior to ovulation (Hoff et al., 1983; Knobil, 1974), but it is not clear what, if any, positive feedback effect progesterone has on the LH surge in gonad-intact females. Progesterone induces an LH surge following a small dose of E2 in ovariectomized female rhesus monkeys by increasing the frequency and amplitude of GnRH release (Terasawa et al., 1980, 1987). However, large doses of estradiol alone are also sufficient for positive feedback to occur (Terasawa et al., 1980, 1987). Thus, progesterone may facilitate positive feedback and the LH surge in females, though it does not appear to be required.

Puberty onset is marked by increased GnRH release from the hypothalamus, whereas the juvenile quiescent period of HPG activity results from inhibition of GnRH release rather than inhibition of pituitary or gonadal function (Knobil, 1980; Plant et al., 1989; Wildt et al., 1980). Administration of a glutamate agonist, N-Methyl-DL-aspartic acid (NMDA), stimulates GnRH release (Mahachoklertwattana et al., 1994). In prepubertal male rhesus macaques (15-16 months of age), intravenous administration of NMDA every 3 hours resulted in increases in LH, FSH, T, and testicular volume after sixteen weeks of treatment not observed in age-matched control males (Gay & Plant, 1987, 1988; Plant et al., 1989). Thus, GnRH was capable of being released and the pituitary was functional and able to respond to the GnRH released by NMDA treatment. Furthermore, the testes were capable of responding to gonadotropin stimulation, even though they were inactive in the untreated monkeys. When NMDA treatment was

discontinued, LH, FSH, and T levels decreased to those observed prior to NMDA treatment, indicating the hormonal increases directly resulted from the GnRH released by NMDA administration (Plant et al., 1989). That the active hormone was GnRH is demonstrated by the finding that a GnRH antagonist blocked the effectiveness of NMDA treatment in increasing LH and FSH (Plant et al., 1989). Thus, the inactivity of the HPG axis during the juvenile period in males likely reflects an inhibition of GnRH release and removal of this inhibition produces an increase in GnRH release, marking the onset of puberty in males.

Similar mechanisms appear to operate in females, even though full female reproduction requires both negative and positive feedback in contrast to the need for only negative feedback in males (Chongthammakun & Terasawa, 1993; Plant & Dubey, 1984; Plant et al., 1978). Lesions of the arcuate nucleus in prepubertal females (11-15 months of age) combined with pulsatile GnRH treatment resulted in increased LH, smaller increases in FSH, and, after 2-4 LH surges luteal levels of progesterone, indicating ovulation had occurred (Knobil, 1980; Wildt et al., 1980). Thus, as in males, the juvenile female pituitary and gonad are developed and capable of responding to GnRH release well before puberty onset is typically observed, indicating that GnRH release is the factor regulating puberty onset. Lastly, levels of the inhibitory neurotransmitter, γ-Aminobutyric acid (GABA), are inversely related to GnRH levels prior to puberty and decline prior to GnRH release (Mitsushima et al., 1994). Bicuculline treatment blocks $GABA_A$ receptor activation and results in increased GnRH release, and chronic treatment results in menarche and first ovulation occurring about one year earlier than control females (Keen et al., 1999; Mitsushima et al., 1994). Blocking GABA inhibition results

in earlier increases in GnRH release and earlier puberty onset, indicating that in females, as in males, there is an inhibition of GnRH release that is removed, resulting in the puberty-initiating increase in GnRH.

In both juvenile males and females, there is an inhibition of GnRH release that is removed prior to the onset of puberty (Gay & Plant, 1987, 1988; Keen et al., 1999; Mitsushima et al., 1994; Plant et al., 1989). Although mechanisms regulating inhibition of GnRH release may differ between males and females (as reviewed by Plant, 2001), the influence of the peptide kisspeptin on GnRH release is one mechanism that appears to be similar in males and females. Increases in kisspeptin release occur prior to the pubertal increase in GnRH release and exogenous kisspeptin administration results in GnRH release in both males and females, which suggests that changes in kisspeptin release influence puberty onset in both males and females (Guerriero et al., 2012; Keen et al., 2008; Shahab et al., 2005). The mechanisms regulating juvenile GnRH inhibition are currently under investigation and a complete discussion of all of these mechanisms is beyond the scope of this review (see Plant, 2001 for a review).

In females, initiation of puberty appears to involve both the removal of inhibition of GnRH release and desensitization of E2 negative feedback (Keen et al., 1999; Mitsushima et al., 1994; Wilson et al., 1986). Prepubertal, socially housed females ovariectomized at 11 months of age did not show increased LH in response to ovariectomy until 24 months of age, indicating the removal of inhibition on GnRH secretion occurred around 24 months of age (Wilson et al., 1986). However, exposing females ovariectomized at 11 months of age to chronic E2 that produced 40-50 pg/ml blood E2 levels delayed post-ovariectomy increases in LH until approximately 30 months

of age. This delayed increase in LH in the face of low-levels of E2 demonstrates decreased E2 sensitivity on negative feedback occurring approximately 5-6 months after activation of the HPG axis (Wilson et al., 1986). Thus, in gonad-intact females living in social groups, this desensitization to E2 negative feedback occurs approximately 5-6 months after the initial increase in GnRH release. It is this desensitization to E2 negative feedback that allows for increases in E2, which are required for E2 positive feedback to occur.

At the start of puberty onset, increased GnRH release results in increased levels of LH and FSH from the pituitary, ultimately resulting in increases in gonadal hormones (Grumbach & Styne, 2003; Knobil, 1974, 1980). In this review, initial increases in LH and estrogens, first appearance of sexual skin swelling (sex swelling), and menarche are all measures used to indicate puberty onset in females, whereas first ovulation, a sustained increase in luteal progesterone, is considered to be reproductive maturation. In male nonhuman primates discussed in this review, puberty onset is defined as initial increases in LH, T and other androgens, and testicular volumes. Data on environmental influences on puberty timing in males are limited to puberty onset and thus, it is not known at this time how environmental factors alter the timing of reproductive maturation in male nonhuman primates.

This sequence of pubertal events and its timing are influenced by environmental factors. In outdoor-housed rhesus macaque females, menarche typically occurs around 2.5 years of age, with first ovulation occurring at either 2.5 or 3.5 years of age (Wilson et al., 1984; Wilson et al., 1986; Zehr et al., 2005). By contrast, females living indoors experience menarche and first ovulation approximately four to six months earlier than do

females living outdoors (Wilson et al., 1988). Outdoor-housed rhesus macaque males typically show increased T levels and testicular volumes, indicating puberty onset, at 3.5 years of age (Herman et al., 2006). Thus, in rhesus macaques as in humans, females enter puberty earlier than do males, with the age at puberty onset varying between individuals, and environmental context accounts for at least some of the variation in puberty timing. Because GnRH is required for gonadal function in both males and females and GnRH release is the rate-limiting factor in puberty onset (Keen et al., 1999; Mitsushima et al., 1994; Plant & Dubey, 1984; Plant et al., 1978; Plant et al., 1989), environmental factors that influence the timing of puberty onset likely alter puberty timing by altering GnRH release. This review discusses the environmental factors that regulate puberty timing, the social and hormonal factors that explain some of the variation in puberty timing between individuals, as well as changes in sexual behavior that occur around puberty. The vast majority of this review focuses on captive rhesus macaques (*Macaca mulatta*) because most of the research on puberty has been done in this species; however, wherever there is evidence from other nonhuman primate species that is included.

Seasonal Influences on Puberty Onset

Males

Species that have distinct breeding seasons in adulthood also have seasonal patterns of puberty onset. For example, outdoor-housed rhesus macaque males at the latitude of Atlanta, GA showed increases in testicular volume and T levels beginning in September and showed decreases in testicular volumes and T levels in January, which continued to decline with testicular volumes becoming the smallest in April, at the end of

the breeding season (Herman et al., 2006). During their first pubertal breeding season, rhesus macaque males showed increased levels of T, but lower levels than in fully adult males. This increase occurs at the same annual time as does the onset of breeding conditions in fully adult males. By a male's second pubertal breeding season, their T levels during the breeding season were similar to those observed in adult males (Bercovitch, 1993; Herman, et al., 2006).

Day length, rather than temperature, appears to regulate seasonal patterns of T and testicular volume in rhesus macaques. Under constant temperature conditions, testicular size increased during short days (8 hr daylight) and decreased in size during long days (16 hr daylight) in individually-housed prepubertal and pubertal males (Chik et al., 1992). T levels paralleled changes in testicular volume, with T increasing along with testicular size during short days and decreasing with testicular size during long days. The effect of day length did not alter testicular size and T levels until animals entered puberty, when testicular diameters were greater than 10mm (Chik et al., 1992), which suggests that day length alters the activity of the HPG axis and the increased release of GnRH beginning at the time of puberty onset.

In species that do not show distinct breeding seasons such as wild savanna baboons (*Papio cynocephalus*), testicular size increases, determined by monthly visual assessment, were not seasonal, occurring in all seasons (Alberts & Altmann, 1995). Thus, male puberty onset is influenced by seasonal factors such as day length, but only in species that have a distinct breeding season in adulthood.

Females

Unlike testicular function in males, menarche in outdoor-housed female rhesus macaques was not restricted to a particular season, occurring at approximately 2.5 years of age, regardless of time of year (Wilson et al., 1986; Wilson et al., 2013). Females born during the spring or summer and females born in the fall or winter all showed menarche around 2.5 years of age (Wilson et al., 1984), but during different seasons. Thus, age at menarche appears to be more tightly linked to chronological age rather than birth season.

In contrast to menarche, first ovulation in spring-born, outdoor-housed female rhesus macaques was restricted to the fall months when females were either 2.5 or 3.5 years of age (Wilson et al., 1986; Wilson et al., 2013; Wilson & Gordon, 1989a; Zehr et al., 2005). Ovulation was not observed in the nonbreeding season (February or March to September) (Wilson et al., 1986; Wilson et al. 2013). Similarly to spring-born females, females born in the fall or winter first ovulated in the fall/winter months and were more likely to reach first ovulation around 3 years of age as opposed to 4 years of age (Wilson & Gordon, 1989a). First birth was limited to the spring and summer months regardless of which season females were born, further demonstrating that first ovulation was restricted to the fall and winter months regardless of chronological age (Wilson et al., 1984). Thus unlike menarche, first ovulation appears to be strongly influenced by season. GnRH release itself is presumably influenced by these seasonal factors as ovariectomized females, whose basal LH is elevated by the removal of E2 negative feedback, also showed decreased LH during the spring and summer (Wilson et al., 1986). This seasonal pattern in LH release is thought likely to reflect a seasonal pattern in GnRH release, which restricts the timing of first ovulation to the fall and winter months. The

mechanism controlling seasonal changes in GnRH release is not known, but possibly reflects day length.

As in males, seasonal changes in female HPG function are correlated with changes in day length. Investigations of the internal signal that transduces changes in day length have focused on melatonin, with some evidence suggesting that melatonin could be that signal. In outdoor-housed female rhesus macaques, daily melatonin injections that mimic short day (~10hr daylight) melatonin patterns were initiated at 22 months of age, which was the start of the nonbreeding season (long days), continued for about 11 months, and resulted in first sexual swelling and menarche five to six months earlier than in control animals (Wilson & Gordon, 1989b). These changes in the melatonin-treated animals occurred during the summer (nonbreeding season), whereas first sexual swelling and menarche occurred during the fall (breeding season) in control animals. Thus, melatonin patterns typically seen during shorter days may influence the timing of puberty onset, but specific melatonin patterns are not required for puberty onset as puberty onset in outdoor-housed female rhesus macaques is not limited to a specific season (Wilson et al., 1984; Wilson et al., 1986; Wilson et al., 2013; Wilson & Gordon, 1989b). First ovulation in all but one melatonin-treated animal occurred at approximately 2.5 years of age, whereas first ovulation occurred at 3.5 years of age in control females, with first ovulation occurring during the breeding season in all females (Wilson & Gordon, 1989b). Thus, exposure to levels of melatonin typically seen during shorter days resulted in earlier menarche and uniformly earlier first ovulation, but the influence of season on first ovulation in melatonin-treated females is not clear. There appears to be a minimum amount of time needed between the initial increase in LH and first ovulation as

desensitization to E2 negative feedback occurs approximately 6 months after the initial increases in LH (Wilson et al., 1986). In melatonin-treated females, first ovulation coincided with the breeding season and occurred approximately 5-6 months after first sexual swelling and menarche (Wilson & Gordon, 1989b), which typically occurs within 1 month of the initial LH increase (Wilson et al., 1986, 1988). Therefore, it is not clear whether the timing of first ovulation in melatonin-treated females was influenced by seasonal factors independent of melatonin levels or if the timing of first ovulation would have occurred about six months after initial increases in LH in these females, regardless of season.

Menarche, first ovulation, and increased serum LH occurred significantly earlier in indoor-housed animals living in small groups exposed to a 12/12 hr light-dark cycle and a consistent temperature in comparison to outdoor-housed animals living in large social groups (Wilson et al., 1988). Living outdoors and exposure to seasonal changes delayed puberty onset and reproductive maturation in females. However, body size (17-26 months of age), skeletal maturation (27-42 months of age), and growth hormone levels (18-36 months of age) were significantly greater in indoor-housed animals in comparison to outdoor-housed animals (Wilson et al, 1988). Thus, exposure to seasonal changes may influence the timing of puberty onset and reproductive maturation by delaying physical growth and development. Regardless of whether females lived indoors or outdoors, first ovulation was restricted to the fall/winter months in all females (Wilson et al., 1988). The mechanism for limiting first ovulation to the fall under such different environmental conditions is not known. As with the melatonin-treated females, it is possible that first ovulation in indoor-housed animals is not influenced by season per se, but rather because

first ovulation occurs approximately 6 months after initial increases in LH (Wilson et al., 1988), first ovulation happens to coincide with the breeding season. Although there appears to be a clear seasonal influence on the timing of first ovulation in outdoor-housed female rhesus macaques, the exact mechanism(s) regulating this seasonal pattern is unknown.

In wild female savanna baboons, which do not exhibit seasonal changes in gonadal function in adulthood, initial sexual swelling was not restricted to a particular season and was observed throughout the year (Bercovitch & Strum, 1993). Menarche occurred approximately 56 days after first sexual swelling, with age at initial sexual swelling and age at menarche being strongly correlated. Thus, menarche was likely not restricted to a particular season (Bercovitch & Strum, 1993). It is not known whether there are seasonal influences on first ovulation in savanna baboons, but considering adult ovarian function is not restricted to a particular season, it seems unlikely that first ovulation would be restricted to a particular season.

Body Weight and Puberty Onset

It has been hypothesized that there is a critical body weight necessary for puberty onset (Frisch, 1972; Frisch & McArthur, 1974). Body weight is thought to influence the timing of puberty onset via leptin, a hormone produced by the *obese/lep* gene that signals satiety and influences fat storage (Pelleymounter et al., 1995). Mice lacking the *obese/lep* gene are infertile and leptin administration to these females restores reproductive function indicating leptin can influence activity of the HPG axis (Chehab et al., 1996). Leptin administration to normal female mice accelerates the timing of puberty onset (Ahima et al., 1997), likely as a result of altered GnRH release. Leptin can increase

the release of GnRH from the hypothalamus, invoke the release of LH and FSH, and act directly on the gonads (Lebrethon et al., 2000; Yu et al., 1997). Leptin was strongly correlated with body fat in girls, with increased body fat and greater leptin levels related to earlier menarche (Matkovic et al., 1997). These results support the importance of body weight on puberty onset and suggest this effect may occur via an increase in leptin. Although leptin levels were positively related to body fat in both girls and boys at all stages, leptin increase occurred before LH and FSH peaks in girls, but was unrelated to LH and FSH levels in boys as LH and FSH continued to rise while leptin decreased (Rutters et al., 2009). The increase in T in boys throughout development appears to negatively affect leptin release (Horlick et al., 2000). Thus in humans, it appears the effect of leptin on puberty onset is limited to girls. Research in nonhuman primates allows for the direct examination of the effects of body weight and leptin on puberty onset and how these effects may differ between males and females.

Males

There is some evidence from rhesus macaques in support of body weight influencing puberty onset. For example, male rhesus macaques starting puberty at 3.5 years of age weighed significantly more at the start of that breeding season than males that reached puberty one year later, at 4.5 years of age (Mann et al., 1998). In addition, body weight and testicular volume were significantly correlated at 3.5 years of age (Bercovitch, 1993; Herman et al., 2006). Although these data support a relationship between body weight and puberty onset in males, T levels were not related to either body weight or testicular weight during the first two breeding seasons (Bercovitch, 1993). Thus, increased body weight is only related to puberty onset and is not related to the

degree of HPG activation during puberty. The mechanism by which body weight may influence puberty onset is not clear as leptin does not appear to directly influence male puberty onset.

In juvenile male rhesus macaques living outdoors in large, social groups, leptin levels peaked at 12 and 22 months of age, well before puberty onset (Mann et al., 2000, 2002). Despite these changes in leptin, these peaks in leptin in juvenile males do not appear to influence puberty onset. Chronic leptin infusion did not alter LH levels in indoor-housed castrated prepubertal males, 16-20 months of age, which suggests that GnRH release was not altered by increased leptin levels (Barker-Gibb et al., 2002). Intravenous GnRH administration increased LH levels comparably during both leptin or vehicle infusion, further supporting that exogenous leptin does not alter GnRH release or alter the timing of puberty onset (Barker-Gibb et al., 2002). Bi-monthly endogenous leptin levels did not change during the year prior to puberty onset (26-38 months of age) or during puberty (39-50 months of age), and leptin levels remained constant even though increases in LH, T and testicular volume occurred between approximately 40-46 months of age in socially-housed male rhesus macaques living outdoors (Mann et al., 2000, 2002). Thus, there are no changes in endogenous leptin levels that seem to coincide with activation of the HPG axis and puberty onset. Although there were no observed changes in leptin levels prior to puberty, these data do not indicate whether differences in leptin levels between individuals account for some of the variation in the timing of puberty onset. In gonad-intact rhesus monkeys, leptin levels from 8-24 months and 26-50 months of age did not differ between males that reached puberty at 3.5 years of age and males that exhibited delayed puberty at 4.5 years of age, which suggests that variation in

puberty timing is not influenced by leptin (Mann et al., 2002). Despite the relationship between body weight and puberty onset, the data indicate that leptin does not influence puberty onset in male rhesus macaques as leptin levels do not change around the time of puberty and differences in puberty timing are not the result of differences in leptin levels. Therefore, it is not clear how body weight may influence puberty onset in male rhesus macaques or if this relationship is simply a consequence of another factor influencing both body weight and puberty onset.

Females

A review of fourteen studies (2-33 females per study) found a decline in age at menarche over a fifty year period (1920s-1970s) in female rhesus macaques (Wilen & Naftolin, 1976). Consistent with this finding, Terasawa and colleagues (2012) found a decline in age at menarche from the 1970s-2000s in females at the Wisconsin National Primate Research Center (N=23), demonstrating a secular trend for earlier menarche. Despite the shift in age at menarche over time in female rhesus macaques, average body weight at menarche did not differ between time periods, supporting the hypothesis that a critical body weight is required for puberty onset (Wilen & Naftolin, 1976). A review of archival data in rhesus monkeys revealed significantly faster increases in body weight in more recent years, 2003-2005, in comparison to time periods between 1988-1990 and 1973-1975 and this significantly faster growth rate was related to earlier age at menarche (Terasawa et al., 2012). Secular trends indicate that puberty onset is occurring earlier as result of earlier weight gain, but these trends do not provide information about how body weight relates to the timing of puberty onset on an individual level and whether body weight accounts for any variation in the timing of puberty onset between individuals.

Further examination of individual archival data revealed that animals reaching menarche or first sexual swelling earlier weighed significantly less at puberty than did animals who reached puberty later. Thus, the hypothesis that a critical body weight is required for puberty onset is only supported at a population level and is not supported when examining individual data (Wilen & Naftolin, 1976). Although a critical body weight is not required for puberty onset, this evidence demonstrates that body weight as well as other growth factors may influence the timing of puberty onset.

In female rhesus macaques living outdoors, age at menarche, a mean age of 2.5 years, was not related to body weight or body mass index at menarche (Zehr et al., 2005). Although body weight at menarche was not related to age at menarche, greater increases in body weight prior to menarche (from 10-16 months of age until 26 months of age) were related to earlier menarche or first sexual swelling (Wilson et al., 2013). Thus, juvenile changes in body weight, rather than absolute body weight, may influence the timing of menarche in outdoor-socially-housed female rhesus macaques. It is possible that physical growth factors other than body weight, such as body length or growth hormone levels, which may also be related to body weight, are more influential in altering the timing of menarche rather than body weight itself. For example, patterns of body weight changes did not differ between indoor-housed and outdoor-housed females, despite earlier menarche in indoor-housed animals (Wilson et al., 1988). Increases in body length (crown-rump length) occurred prior to menarche in both indoor- and outdoor-housed females, however, indoor-housed females experienced earlier increases in body length (17-26 months of age) and greater growth hormone levels (18-36 months

of age) in comparison to outdoor-housed females, indicating that skeletal growth and/or growth hormone levels may influence age at puberty onset (Wilson et al., 1988).

Despite no relationship between body weight at menarche and age at menarche, differences in body weight and body mass index were related to the timing of first ovulation. Females that experienced first ovulation during the same season when menarche occurred, at approximately 2.5 years of age, weighed more and had a higher body mass index at 24 and 30 months of age than did females who experienced first ovulation a year later, at approximately 3.5 years of age (Zehr et al., 2005). Therefore, earlier age at first ovulation may be influenced by greater body weight and body mass index at the time of menarche (Zehr et al., 2005). Females that did not ovulate until 3.5 years of age had the greatest increase in body weight and growth hormone concentrations in the summer months, when 3 years of age, between menarche and first ovulation, demonstrating that body weight gain occurred prior to first ovulation (Wilson et al., 1984). However, differences in body weight at menarche or first ovulation are not sufficient to explain this variation in age at first ovulation as Wilson and colleagues (1986) found this variation in age at first ovulation, despite no differences in body weight at or prior to 31 months of age. Changes in growth hormone levels may also account for some of the variation in age at first ovulation. Increases in growth hormone were greater at 20-25 months of age and 26-31 months of age in females that experienced first ovulation at 2.5 years of age in comparison to females that experienced first ovulation at 3.5 years of age. Increases in growth hormone levels were observed approximately one year later in females that reached first ovulation at 3.5 years of age in comparison to females that reached first ovulation at 2.5 years of age, suggesting that growth hormone

levels may also be associated with the variation in puberty timing (Wilson et al., 1986). Though body weight, body mass index, and growth hormone levels may explain some of the variation in the timing of puberty onset, it is likely that multiple growth factors interact to influence the timing of puberty onset.

Based on the data showing that developmental growth factors influence the timing of puberty onset, research manipulating the developmental growth patterns by increasing the fat content of food resulted in earlier puberty onset in females (Schwartz et al., 1988; Terasawa et al., 2012). Consumption of the higher-calorie diet resulted in first sexual swelling and menarche approximately 4-6 months earlier, and more females fed the high-fat diet ovulated at 2.5 years of age (Schwartz et al., 1988; Terasawa et al., 2012). This earlier puberty onset is not easily accounted for by body weight or body mass measures. Although Terasawa and colleagues' indoor-housed females fed the high-fat diet had greater body weight and height than did control females, Schwartz and colleagues' outdoor-housed females fed the high-fat diet weighed significantly less than did control females at menarche. Overall rates of weight and growth increases did not differ between females fed a high-fat diet and females fed the control diet until 27-33 months of age, at which time control animals weighed more than females fed the high-fat diet (Schwartz et al., 1988). Whether the fact that Terasawa's females were indoor-housed while Schwartz's females were outdoor-housed altered the relationship between weight and puberty onset is not known. It is clear, however, that the effect of a high-fat diet on puberty onset is not clearly associated with increases in body weight; rather, changes in body weight may be a proxy for caloric flux, which may be what is influencing puberty onset (as reviewed by Schneider et al., 2012). Terasawa and colleagues found the high-

fat diet resulted in a greater body mass index and abdominal fat measures, as well as greater leptin levels. In contrast, Schwartz and colleagues found that consumption of a high-fat diet resulted in lower body fat at the time of menarche, but this difference in body fat did not differ by treatment at the time of first ovulation. Although earlier puberty onset with a high-fat diet was found in both studies, the data clearly show that body weight or body fat are not determinative and are at best a correlate of the factors that regulate puberty onset. Something like energy availability, which may correlate under some conditions with body weight and not under other conditions (e.g. of high energy expenditure), could be the common mechanism by which puberty onset varies.

In gonad-intact, indoor-housed female rhesus macaques, both diurnal and nocturnal leptin levels were significantly higher at the age when the nocturnal rise in LH was detected, an early sign of activation of the HPG axis, in comparison to leptin levels prior to the nocturnal rise in LH (Wilson et al., 2003). Leptin administration from 12-30 months of age hastened the nocturnal rise in LH, sexual swelling, age at menarche, and age when E2 levels became detectable, but this was not accompanied by differences in body weight between leptin-treated and control females. Although leptin administration resulted in earlier puberty onset, it did not influence the timing of first ovulation in indoor-housed females (Wilson et al., 2003). Serum leptin levels were significantly lower in outdoor-housed, low-ranking females from 24-30 months of age in comparison to high-ranking females (Wilson & Kinkead, 2008). This effect of decreased leptin levels in low-ranking females was due to lower leptin levels from 24-27 months of age in subordinate females with a specific genetic polymorphism. Low-ranking females with at least one allele coding for a short promoter region, 5HTTLPR, on the serotonin

transporter gene, SLC6A4, experienced later first ovulation in comparison to all high-ranking females, regardless of the presence of this polymorphism, and low-ranking females without this polymorphism, indicating that the effects of leptin on first ovulation are likely influenced by both genetic and social factors (Wilson & Kinkead, 2008).

In summary, in males, body weight bears no clear relationship to gonadal function and leptin has no influence on the timing of puberty onset. In contrast, in females, body weight, body mass, physical body growth, and diet content can be related to the timing of puberty onset, though differences in the timing of puberty onset are not always related to these growth factors. Leptin and growth hormone concentrations are two potential mechanisms by which body growth influences the timing of puberty onset in females.

Social Rank and Puberty Onset

Males

In wild male savanna baboons living in multi-male/multi-female groups, the onset of puberty is characterized by increases in testicular size and the end of the pubertal transition to adulthood is marked by dispersal from the natal group or attaining status within the adult male hierarchy of the natal group (Alberts & Altmann, 1995). Prior to developing their own social rank, male savanna baboons inherit their natal group rank from their mothers and this is the male's rank at the time of puberty onset. Maternal rank was strongly related to increases in testicular size as higher-ranking male savanna baboons showed earlier increases in testicular size than did lower-ranking males (Alberts & Altmann, 1995; Charpentier et al., 2008). A similar relationship between maternal rank and puberty onset was found in male mandrills (Setchell et al., 2006). Maternal social rank was similarly related to the age when males attained adult social rank (Alberts

& Altmann, 1995), with males from higher maternal ranks attaining adult social rank earlier that did males from lower-ranking mothers. This suggests that maternal rank is also related to the timing of the culmination of puberty in male savanna baboons. If high social rank results in earlier testicular development as well as shortens the period of time between testicular development and age of first adult rank, then it is possible that maternal rank influences these two factors independently. However, if the length of puberty in males does not vary by maternal rank, then that would suggest that maternal rank influences age of puberty onset and the relationship between maternal rank and age at first adult rank is simply a result of earlier puberty onset in higher-ranking males. Although the influence of maternal social rank on factors occurring later in the pubertal period is not clear, it is clear that higher maternal social rank is related to earlier testicular development in males.

As in wild savanna baboons, in male rhesus macaques living in multi-female/one-male groups, maternal social rank was related to both testosterone levels and testicular weight during the breeding season when males were approximately 3.5 years of age (Dixson & Nevison, 1997). Male offspring from higher-ranking mothers had both higher T levels and greater testicular weights (Dixson & Nevison, 1997), which is consistent with the data from savanna baboons showing high maternal rank was related to earlier testicular growth (Alberts & Altmann, 1995). T levels and testicular weight were also correlated, with higher T levels being related to greater testicular weight, which suggests testicular weight is a valid proxy for T levels in adolescent males when blood collection is not possible (Dixson & Nevison, 1997). Though maternal rank appears to influence aspects of pubertal development such as T levels and testicular weight, this study was

limited to a single season of data collection and does not provide data on whether maternal rank is related to the timing of puberty onset.

Around the time of puberty onset, male rhesus macaques remaining in their natal group are integrated into the linear male hierarchy (Koford, 1963) and thus, rank within the male hierarchy may also influence the timing of puberty. In male rhesus macaques living outdoors in large, multi-male/multi-female groups, social rank within the male hierarchy was not related to maximum testicular volume or increases in serum LH and T following a dose of exogenous GnRH at 3 years of age, prior to the first pubertal breeding season (Herman et al., 2006). However, by 3.5 years of age, the age of puberty onset in male rhesus macaques, social rank was related to maximum testicular volume, mean serum T and LH levels (September-January), and responsiveness of the HPG axis (Bercovitch, 1993; Herman et al., 2006). At 3.5 years of age, higher-ranking males had larger testicular volumes, higher endogenous serum T and LH levels, and higher T and LH levels in response to an injection of GnRH, which suggests that during the first pubertal breeding season, higher-ranking males experienced greater activation of the HPG axis in comparison to lower-ranking males (Bercovitch, 1993; Herman et al., 2006).

During the nonbreeding season at 4 years of age, social rank continued to be related to testicular volume, but not to serum T and LH levels (Herman et al., 2006). Despite the lack of a relationship between social rank and T or LH levels at 4 years of age, increases in T following a GnRH injection remained related to social rank, and there was a trend for a relationship between social rank and increases in LH following exogenous GnRH. Thus, social rank is still related to an increased responsiveness of the

HPG axis at 4 years of age, but this effect is not naturally observed likely due to lower levels of circulating T and LH during the nonbreeding season (Herman et al., 2006).

During the second pubertal breeding season, 4.5 years of age, social rank remained related to testicular volume, there was trend for a relationship between social rank and serum LH, but there was no relationship between social rank and serum T (Herman et al., 2006). Consistent with these findings, there was a trend for a relationship between social rank and LH response to exogenous GnRH, but there was no relationship between social rank and T response to exogenous GnRH. Thus, at 4.5 years of age, the trend for a relationship between social rank and LH levels or LH response to GnRH suggests that higher-ranking animals are producing more LH, but that this increase in LH is not resulting in increased T levels. In summary, in male rhesus macaques, social rank is related to greater responsiveness of the HPG axis at 3.5 and 4 years of age, but this effect disappears, with the exception of differences in testicular volume, by 4.5 years of age (Herman et al., 2006).

Bercovitch and colleagues (1993) found that high-social rank predicted earlier seasonal increases in T in pubertal males and lower-social rank was associated with delayed seasonal increases in T, but peak T levels during the breeding season did not vary with rank. Thus, the effects of social rank in male rhesus macaques are likely limited to the initial increases in T during the breeding season. The mechanism(s) regulating the relationship between social rank and activation of the HPG axis are not clear, but this effect of social rank is likely not mediated by greater body weight in high-ranking animals as there was no relationship between social rank and body weight, despite

relationships between testicular volume and both social rank and body weight (Bercovitch, 1993; Mann et al., 1998; Herman et al., 2006).

Females

Female social rank was related to age at puberty onset in savanna baboons with dominant females having an earlier age at first sexual swelling and menarche (Bercovitch & Strum, 1993; Charpentier et al., 2008). Age at first sexual swelling was positively correlated with age at menarche and menarche occurred, on average, two months after first sexual swelling (Bercovitch & Strum, 1993). The interval between first sexual swelling and menarche did not differ between high and low-ranking females (Bercovitch & Strum, 1993), which suggests that the influence of social rank is limited to puberty onset and once puberty is initiated, social rank does not further predict the timing of pubertal markers. Interestingly, age at first birth did not vary by social rank, suggesting that earlier puberty onset does not result in greater reproductive success (Bercovitch & Strum, 1993). Family and group structure also appear to influence the timing of menarche in savanna baboons as earlier menarche was associated with more adult female siblings as well as fewer female group members (Charpentier et al., 2008).

In contrast to savanna baboons, in female rhesus macaques, social rank was not related to age at first sexual swelling (Wilson & Kinkead, 2008) or age at menarche when all females reached menarche around 2.5 years of age (Wilson & Kinkead, 2008; Zehr et al., 2005). The lack of association between female social rank and puberty onset may reflect the lower variation in age of menarche in rhesus macaques than in savanna baboons (Bercovitch & Strum, 1993; Wilson & Kinkead, 2008; Zehr et al., 2005). These findings need to be interpreted cautiously as higher social rank was found to be

associated with earlier puberty onset when females were studied year round beginning at approximately 1 year of age and when puberty onset is defined as either menarche or first sexual swelling (Wilson et al., 2013). The exact relationship between sexual swelling and menarche in female rhesus macaques is unknown, but both are likely to reflect underlying neuroendocrine events; however, whether the events they reflect are the same for both endpoints is currently unknown. At this point it is unclear whether higher social rank predicts earlier menarche.

Age at first ovulation, however was clearly related to social rank with higher-ranking females reaching first ovulation at an earlier age (Wilson et al., 2013). When social rank (high, middle, and low) and age at first ovulation (2.5 or 3.5 years of age) were grouped for analysis, high- and middle-ranking females first ovulated at either 2.5 or 3.5 years of age, whereas all low-ranking females experienced first ovulation at 3.5 years of age (Zehr et al., 2005). These data demonstrate that in female rhesus macaques, higher social rank does not uniformly predict earlier first ovulation, but rather low-social rank delays first ovulation. Although age at first ovulation varied by social rank, there were no differences in body weight at first ovulation based on social rank (high, middle, low) indicating that differences in body weight at 2.5 years of age may explain the variation in the timing of first ovulation (Zehr et al., 2005). Females that first ovulated at 2.5 years of age had greater body weights and higher body masses at this age in comparison to the body weights and body masses at 2.5 years of age of females that ovulated a year later. In addition, low-ranking females weighed significantly less than high- or middle-ranking females at 2.5 years of age. It is possible that age at first ovulation is regulated primarily by body weight and that lower-ranking females may

ovulate later due to lower body weights at the time of menarche (Zehr et al., 2005). However, it is equally likely that delayed puberty and lower body weight are both a consequence of low social rank and that weight per se is not the cause of the delayed puberty in lower-ranking females. Wilson and Kinkead (2008) found no social rank differences in weight gain between birth and 2.5 years of age despite finding delayed ovulation in low-ranking females, which supports the idea that the effect of social rank on first ovulation is not mediated through differences in body weight *per se* but rather that social rank may influence body weight and puberty onset independently.

Puberty onset, defined as menarche or first sexual swelling, was related to age at first ovulation, and the time between puberty onset and first ovulation was related to age at first ovulation, suggesting that the period of adolescent sterility between puberty onset and first ovulation was shorter in females that ovulated earlier (Wilson et al., 2013). Distinct behavioral experiences as a juvenile (10-16 months until 26 months of age) predicted age at puberty onset and age at first ovulation in female rhesus macaques, which suggests that early social experience may influence puberty timing (Wilson et al., 2013). Juvenile females that received more submissive gestures from members of the group, which would be characteristic of higher-ranking females, reached menarche or first sexual swelling earlier than females receiving less submissive gestures from the group. Less aggression received from group members, less submissive behavior directed towards others, higher rates of affiliation towards others, and greater weight gain from 10-16 months until 26 months of age, which are all consistent with behaviors observed in higher-ranking animals, predicted earlier first ovulation. Lastly, showing higher levels of submission to other group members, which is characteristic of lower-ranking females,

predicted a longer duration between puberty onset and first ovulation, indicating that behaviors characteristic of low-ranking females resulted in a longer duration of puberty (Wilson et al., 2013). In summary, behaviors consistent with high social rank predicted earlier puberty onset and first ovulation, whereas behaviors consistent with low social rank predicted a longer duration of puberty. Although the data demonstrate that early social experience may predict puberty timing, it is not clear whether social rank or behavioral patterns are predicting puberty timing. It is possible that distinct behavioral patterns influence an animal's social rank and thus, behavioral patterns are influencing the differences in puberty timing. Alternatively, it is possible that social rank influences behavioral patterns and social rank is the factor influencing puberty timing.

The influence of social rank is more pronounced in new world monkeys than that seen in old world primates. In adult female common marmosets (*Callithrix jacchus*), a pair-bonding new world primate, dominant females suppress ovulation in subordinate females and this suppression continues as long as the subordinate female is exposed to the scent of the dominant female (Abbott et al., 1988; Barrett et al., 1990). This ovulation suppression does not likely reflect increased stress in the subordinate female, as circulating levels of cortisol are not higher in subordinate females (Abbott et al., 1981). Additional research shows ovulation suppression in adult female marmosets is not a result of decreased hypothalamic GnRH release in subordinate females as GnRH release in breeding females in the follicular phase was similar to GnRH release in subordinate females (Abbott et al., 1997). Thus, in the case of marmosets, social context appears to be influencing the pituitary and/or the ovary, rather than the hypothalamus, which

suggests that in juvenile females this suppression prevents first ovulation, but not puberty onset.

Captive female common marmosets first ovulated at approximately 400 days (13months) of age, but this coincided with removal from the natal group and introduction to a new group. Therefore, it is not clear whether first ovulation occurred as a result of reaching a particular age or from changes in the social context (Abbott & Hearn, 1978). When remaining in the natal group, about 46% of subordinate females ovulated at least once, indicating that first ovulation can occur in the natal group, and females that do not show behavioral submission to their mothers are more likely to ovulate in the natal group (Saltzman et al., 1997, 2004; Sousa et al., 2005). Removal of the male and introducing a new male to the group significantly increased the proportion of females ovulating while in their natal group; however, when accounting for mother-daughter dominance relationships in the analysis, introducing a new male to the group did not significantly increase the number of ovulating females (Saltzman et al., 1997, 2004). Thus, the data suggest social subordination in the natal group suppresses ovarian function in that daughters that are behaviorally subordinate to their mothers are less likely to ovulate than less subordinate daughters.

In contrast to ovarian suppression, suppression of sexual behavior in captive female common marmosets in the natal group appears to be influenced by the presence of the father (Saltzman et al., 2004). Captive female marmosets in their natal group did not engage in sexual behavior, but when the father was removed and an unfamiliar male was introduced, daughters showed an increase in sexual behavior towards the new male. Both anovulatory and ovulatory females showed increased sexual behavior towards the new

male, but females having ovulatory cycles during the behavioral collection period showed a greater increase in sexual behavior than did anovulatory females (Saltzman et al., 2004). Thus, suppression of ovarian function and sexual behavior appear to be regulated by different mechanisms in captive female common marmosets.

In wild common marmosets, ovarian suppression was not observed in the subordinate female, but rather it appeared that sexual behavior was influenced by social rank (Sousa et al., 2005). The dominant female mated exclusively with the breeding male, whereas subordinate females may have attempted to mate with the breeding male, but extragroup copulations were also observed (Sousa et al., 2005). These data suggest that mating attempts by subordinate females, not ovarian function in these females, are largely regulated by the dominant female in wild common marmosets. Thus, suppression or regulation of sexual behavior appears to occur in both wild and captive female common marmosets; however, ovarian suppression may be a consequence of living in captivity.

In captive female Wied's black tufted-ear marmosets (*Callithrix kuhli*), urinary progesterone metabolites and LH levels were low in females less than one year of age, indicating the HPG axis was not activated (Smith et al., 1997). Spontaneous ovulation in females living in their natal group occurred at approximately 15.6 months of age, but these young females had shorter luteal phases accompanied by lower levels of progesterone metabolites in comparison to when housed alone. Young females living in their natal group also had significantly lower progesterone levels than adult breeding females and their luteal phase length also showed a tendency to be longer. Length of the luteal phase did not differ between young females that were housed alone and adult

breeding females. Despite the occurrence of spontaneous ovulation in this species, remaining in the natal group appears to influence ovarian function in the luteal phase in young females (Smith et al., 1997).

In cotton-top tamarins (*Saguinus oedipus*), a pair-bonding species, female offspring living with their family begin showing elevations of urinary LH and estrogens between 15 and 17 months of age, indicating activation of the HPG axis and puberty onset (Ziegler et al., 1987). However, these females did not show cyclic hormonal changes and had significantly lower levels of LH and urinary estrogen conjugates than did the dominant female, their mother. By 26-29 months of age, daughters still showed suppressed ovarian function if they remained in the natal group. Removing a daughter from her family group and housing the female in isolation, preventing visual and olfactory cues from others, did not result in ovulation, indicating removal from the family group is not sufficient to induce ovulation after ovarian suppression. Although urinary estrogen conjugates and LH levels increased in individually-housed females when exposed to auditory and olfactory cues from others, ovulation did not occur until the female was paired with a novel male. Thus, exposure to a novel male is required for adult ovarian function in young, ovarian-suppressed female tamarins (Ziegler et al., 1987).

Removal of a daughter from the family unit prior to puberty onset, at 9 months of age, followed by exposure to an unfamiliar male accelerated puberty onset in a female tamarin and elevated urinary LH and estrogen conjugates by 10 months of age, 5-7 months earlier than was seen in the family unit (Ziegler et al., 1987). It is not known whether introduction to the novel male at 9 months of age also resulted in earlier

ovulation, as a result of the observed earlier increases in LH and estrogen conjugates, or if age at first ovulation was unaffected by this earlier activation of the HPG axis.

In species such as marmosets and tamarins that live in family groups, social context may delay first ovulation, but it is not clear how it affects the timing of puberty onset. It is difficult to determine whether puberty onset, as indicated by increased LH and estrogens, is suppressed in daughters living in a family group, accelerated in daughters removed from the family group and introduced to a novel male, or whether both acceleration and suppression of puberty onset occur in these species.

The effect of gonadal suppression due to remaining in the natal group is limited to females as captive male cotton-top tamarins living in their natal group had urinary LH, T, and dihydrotestosterone (DHT) levels that were comparable to their father, the breeding male in the group (Ginther et al., 2001, 2002). Thus, sons living in the natal group were capable of producing offspring, but mating attempts with females, either their mother or sisters, were rare and mounts were directed towards other males in the group (Ginther et al., 2001). Similar to male cotton-top tamarins, captive male golden lion tamarins (*Leontopithecus rosalia*) living in their natal group did not show decreased fecal androgen levels in comparison to their father (Bales et al., 2006). In addition to tamarins, captive male common marmosets showed increases in testicular volume and plasma T levels around 250 days (8 months) of age, while in the natal group, and their T levels were comparable to those observed in adult males (Abbott & Hearn, 1978). Similarly, levels of urinary androgens in white-faced marmosets (*Callithrix geoffroyi*) increased throughout development and by 16-24 months of age, urinary androgen levels of sons living in the natal group did not differ from their father, the breeding male in the group

(Birnie et al., 2011). Thus, in tamarins and marmosets, males appear to go through puberty in the natal group without any evidence of gonadal suppression. However, male marmosets and tamarins have not been subjected to as many social manipulations as have females, so we don't know for certain that pubertal events are not sensitive to social context in sons as they are in daughters.

Despite a lack of testicular suppression in their natal group, testicular suppression has been observed in subordinate golden lion tamarins unrelated to the dominant male (Bales et al., 2006). However, such testicular suppression was not observed if the subordinate male was related to the dominant male, suggesting that gonadal suppression can occur in some social contexts in males, but may depend on the relationship or interactions between the pair of males. Thus, both males and females show gonadal suppression but the social contexts in which this occurs may differ by sex.

Pre- and Neonatal Testosterone Levels and Puberty Onset

A sex difference in the age at puberty onset exists in humans in that girls enter puberty earlier than boys (Marshall & Tanner, 1969; 1970). This sex difference in puberty onset has also been documented in some nonhuman primate species, such as rhesus macaques (Zehr et al., 2005; Herman et al., 2006). One potential hypothesis for this sex difference is that elevated pre- and neonatal testosterone organizes the HPG axis in males such that early androgen exposure delays puberty onset in males in comparison to females. This section reviews the evidence on the influence of pre- and neonatal testosterone exposure and the relationship to puberty onset.

Males

Exposing fetal rhesus macaque males to exogenous prenatal T for 30-35 days either early (beginning between gestational days 35-40) or late (beginning between gestational days 110-115) in a 170 day gestation had no effect on testicular volumes, T levels, or LH levels in comparison to control males between 3.5 and 4.5 years of age (Herman et al., 2006). However, the dose of T used (20mg/kg testosterone cypionate per week) was not particularly large as it was insufficient to masculinize the genitals of female fetuses exposed to this androgen treatment (Herman et al., 2000). Thus, it is possible that because of the negative feedback effects of exogenous T, which would reduce endogenous androgens, that the fetal males were not actually exposed to elevated levels of androgens.

In contrast to the lack of effects of prenatal androgen treatment, males treated prenatally with flutamide, an androgen receptor blocker, early in gestation had significantly larger testicular volumes and higher T and LH levels at 3.5 years of age in comparison to control males, who did not show comparable testicular volumes and T and LH levels until 4.5 years of age (Herman et al., 2006), suggesting earlier puberty onset. Interestingly the genitals of these early flutamide-treated males were significantly more female-like and less masculinized than those of control males and these males also showed a female-typical earlier puberty onset (Herman et al., 2000; Herman et al., 2006). This finding is consistent with prenatal androgen organizing the later puberty onset in males compared to females.

In contrast to its effects when administered early in gestation, flutamide administered late in gestation did not result in higher levels of T or LH at 3.5 years of age, although testicular volumes were significantly larger than in control males (Herman

et al., 2006). The source of the increased testicular volume is not known, but suggests the effects of prenatal flutamide exposure on the organization and later functioning of the HPG axis are limited to early gestation.

By 4.5 years of age, there were no longer any differences between prenatally flutamide-treated and control males (Herman et al., 2006). Thus, flutamide treatment specifically affected the timing of puberty onset and did not produce subsequent differences in HPG function. Although a number of critical experiments remain to be done, the most consistent explanation is that elevated prenatal T early in gestation delays puberty and blocking this elevation advances puberty onset in males.

In addition to the prenatal activation of the HPG axis, males show a neonatal increase in T during the first three months of life (Mann et al., 1984, 1989). Neonatal suppression of the HPG axis in males using a GnRH agonist (D-Trp^{6}-N-α-Me-Leu^{7}-des-Gly^{10}-Pro^{9}-NHEt-GnRH) for 112 days beginning at 10-13 days of age suppressed this neonatal T surge and resulted in blunted T levels during the pubertal period in male rhesus macaques (Mann et al., 1989, 1993). At 3 years of age, GnRH-agonist-treated males had significantly lower T levels before and in response to an intravenous injection of GnRH in comparison to control males, though the pattern of increase in T was similar in both control and treated animals. However, serum LH levels in response to a GnRH injection did not differ by treatment (Mann et al., 1989). These data suggest that neonatal GnRH-agonist exposure, which suppressed neonatal T, resulted in decreased testicular sensitivity to LH or that the testicles were less developed and thus, produced less T in response to the increased LH levels. It is possible that differences in LH were not observed because samples were collected monthly and that more frequent sampling may

indicate lower serum LH levels in GnRH-agonist-treated animals. At 3.5 years of age, GnRH-agonist-treated males had blunted T levels, lower testicular volumes, and lower sperm counts in comparison to control males, despite no apparent differences in LH levels (Mann et al., 1989, 1993). Half of the neonatally GnRH-agonist-treated animals failed to show an increase in serum T or testicular volumes at 3.5 years of age, likely a result of lower serum LH levels in these males in comparison to GnRH-agonist-treated animals that showed increases in T or testicular volumes (Mann et al., 1989, 1993). Thus, GnRH-agonist treatment resulted in delayed testicular development, which suggests that neonatal T exposure organizes the HPG axis and influences the sensitivity of the testes, which interferes with the timing of puberty onset. Differences in serum LH and T as well as testicular volumes between GnRH-agonist-treated males and control males disappeared by 5.5 years of age, indicating the delayed development resulting from suppression of neonatal T did not permanently alter the functioning of the HPG axis and treatment differences were limited to the timing of puberty onset (Mann et al., 1993). GnRH-agonist treatment did not significantly influence body weight at puberty and therefore, cannot account for the observed effects (Mann et al., 1989). Interfering with neonatal T resulted in delayed puberty onset (Mann et al., 1989, 1993), the male-typical pattern, whereas blocking prenatal T's effects resulted in earlier puberty onset (Herman et al., 2006), the female-typical pattern. Thus, T appears to have opposing effects depending on the developmental timing of exposure to T.

GnRH agonists produce an initial large increase in FSH, LH, and T (Akhtar et al., 1983; Heber et al., 1984). Possibly, these elevated hormones were the mechanism by which puberty onset was delayed. GnRH antagonists don't produce this initial hormonal

increase. To investigate the effect of complete neonatal T suppression, rhesus macaque males were treated with vehicle, GnRH antagonist (antide), or antide + T beginning during the first two days of life and continuing through 4 months of age (Mann et al., 1998). The T treatment was designed to be a replacement dosage, but ended up producing higher T levels than in controls and prolonging the neonatal T elevation to 4 months instead of the 3 month endogenous T increase. Males first showed increases in testicular volume at 3.5 years of age, but pubertal increases in T and LH occurred at either 3.5 or 4.5 years of age, signaling puberty. Males reaching puberty at 3.5 years of age did not differ by treatment in LH levels, T levels, or testicular volumes. However, significantly fewer antide- or antide + T-treated males had puberty onset at 3.5 years of age compared to control males and this delayed puberty onset was not related to body weight or social rank (Mann et al., 1998). Thus, antide treatment, regardless of whether it was combined with T treatment, resulted in significantly more males experiencing later puberty onset, which supports neonatal organization of the timing of puberty onset that may involve GnRH release, or androgens, or other factors. However, another possibility is that antide treatment altered the development of immune function and that this in turn delayed puberty onset as both antide treatment and the combined antide plus T treatment altered responsiveness of the immune system (Mann et al., 1994, 1999).

Whether such immune system changes affect puberty timing cannot be reconciled without further research specifically investigating this question. However, both neonatal GnRH-agonist and -antagonist treatment, as well as the combined antagonist plus T treatment, resulted in more males experiencing later puberty onset and altered immune function (Mann et al., 1994). However, in males that reached puberty at 3.5 years of age,

those treated neonatally with a GnRH antagonist did not have different T levels than did controls, whereas neonatally GnRH-agonist-treated males had blunted pubertal T levels (Mann et al., 1989, 1993, 1998). Thus, the initial hormonal elevations produced by neonatal GnRH-agonist treatment may alter the sensitivity of the pituitary and/or the gonad to hormones, thereby influencing negative feedback, or treatment may simply result in delayed testicular development. Although the mechanisms operating during the neonatal period remain unresolved, evidence strongly supports that the early neonatal period is a sensitive period for hormones or immune factors affecting the timing of puberty onset.

Females

In contrast to male rhesus monkeys, in female rhesus monkeys, manipulating prenatal androgen exposure did not alter the timing of puberty. Prenatal exposure to androgen or flutamide, an androgen receptor blocker, for 30 days either early (beginning at day 35-40) or late in gestation (beginning at day 110-115) did not influence the timing of menarche or first ovulation in female rhesus macaques (Zehr et al., 2005). Previous work in rhesus monkeys reported later menarche in females exposed to very large amounts of prenatal androgen early in gestation (Goy et al., 1988). However, the genitals of Goy's early-androgen-treated females were almost completely masculinized and had no vaginal opening, precluding detecting menarche using vaginal swabs as was used by Zehr and colleagues (2005). In the Goy study, menstrual blood exited through androgenized-female's penile urethra and thus, it is unlikely the light bleeding typical of menarche would be detected, but rather only the heavier bleeding typical of menstruation following ovulation would be detected. Thus, the later menarche in the Goy study may

actually reflect first ovulation. Unfortunately, first ovulation was not measured in the Goy study so it unknown what the delayed menarcheal bleeding represents. It is possible that the much higher exogenous T used in Goy's study actually delayed menarche, or equally plausible is that true menarcheal bleeding was not detected.

Further evidence of sex differences in the sensitivity of timing mechanisms come from the finding that neonatal suppression of gonadal activity in female rhesus macaques using a GnRH agonist, Lupron depot, from birth to approximately eight months of age, did not affect age at menarche, age at first ovulation, or the time interval between menarche and first ovulation (Wilson & Kinkead, 2008). Taken together these data suggest that unlike in males, in females, the HPG axis is not organized during prenatal or neonatal time periods and that hormonal manipulations at these times do not alter the timing of pubertal events. Thus, the sex difference in the timing of puberty appears to reflect androgens both pre- and post-natally lengthening the time to puberty in males above that seen in females. Why these neuroendocrine mechanisms are sensitive to prenatal or neonatal androgens in males, but not females is not known.

Pubertal Changes in Behavior

Males

Male macaques display mounting behavior typically within the first three months of life, but these mounts are not accompanied by intromissions or ejaculatory reflexes (as reviewed by Wallen, 1996, 2005). However, there is some evidence to support that full copulatory behavior occurs before endocrine puberty. Male stumptail macaques (*Macaca arctoides*), who mount frequently as young juveniles, began showing intromissions around 1.5 years of age, with the first observed ejaculatory reflex occurring shortly

thereafter, though first seminal fluid emission did not accompany the ejaculatory reflex until approximately four years of age (Nieuwenhuijsen et al., 1988). At 1.5 years of age, behavior was not related to body weight or maternal dominance rank. Thus, juvenile stumptail males have the capacity for full copulatory behavior prior to puberty onset, despite the endogenous suppression of the HPG axis and lack of gonadal hormones. Copulation frequency did not significantly increase until about one year prior to testicular descent and was not related to body weight, T levels, or maternal dominance rank. Thus, an increase in copulatory behavior occurred prior to puberty onset, indicating that the development of the capacity to copulate occurs independently of and is not dependent on gonadal maturation (Nieuwenhuijsen et al., 1988).

Evidence in rhesus macaques also demonstrates the capacity to copulate develops independent of endocrine and behavioral puberty (Wallen, 2001). The components of male sexual behavior (erection, mounting, and intromission) are present well before endocrine puberty. However, the frequency of occurrence of each of these components, as well as the ejaculatory reflex, increases concurrently with endocrine puberty (Wallen, 2001). Ejaculatory reflexes have rarely been reported in male rhesus macaques prior to puberty, but from studies of long-term castrated males we know that T is not required for expression of the ejaculatory reflex (Chambers and Phoenix, 1983; Phoenix et al., 1973). Although the expression of the ejaculatory reflex doesn't require T, suppressing endogenous T reduces ejaculatory frequencies, likely due to decreased sexual motivation (Wallen et al., 1991).

Though endocrine puberty is not necessary for males to express the components of male sexual behavior, the frequency of expression and the sex copulatory behavior is

directed towards changes with puberty. Male rhesus macaques routinely display the stereotyped double-foot-clasp mount used in adult copulation throughout the juvenile period, mounting male and female partners equally (Figure 2; Wallen, 2001). During the peripubertal period male mount rate more than triples, with the increase resulting from an almost 8 fold increase in mounting of female partners (Figure 2). This transition from not discriminating the sex of the mounting partner to almost exclusive mounting of females is best predicted by whether a male was observed to display an ejaculatory reflex with a female, whether or not he had shown a pubertal increase in T (Figure 3; Wallen, 2001). Although most males had increased T prior to their transition to exclusively mounting females, one male who did not have elevated T showed both the ejaculatory reflex and increased mounting of females (Wallen, 2001). Though pubertal increases in T likely increase the probability that males will become sexually involved with females, it is apparent that increased T is not obligatory for this change in male sexual behavior. Part of the function of pubertal T increases is to offset the effects of social context on male sexuality.

At 3.5 years of age, during their first pubertal breeding season, higher rates of mounting with adult and pubertal females was related to higher social rank, larger testicular volume, greater T levels, and greater LH levels in male rhesus macaques (Herman, et al., 2006). During the second pubertal breeding season, 4.5 years of age, mounting rate was still related to social rank, but was not related to T or testicular volume (Herman et al., 2006). This restriction of a relationship between LH levels, T levels, and testicular volume to mounting rate suggests that males showing greater activation of the HPG axis at 3.5 years of age are showing higher rates of mounting. By 4.5 years of age,

rank related differences in T and LH no longer exist, indicating the influence of social rank on sexual behavior is not related to differences in activation of the HPG axis (Herman et al., 2006). Social rank begins exhibiting an influence on sexual behavior at puberty and continues to influence mounting rate throughout puberty. By contrast, social rank was unrelated to the rate of masturbation throughout puberty, indicating the effects of social rank on mounting rates do not reflect differences in sexual motivation (Herman et al., 2006).

One possible reason that social rank affects male sexual behavior is that females selectively avoid mating with low-ranking pubertal males and thus, only high-ranking pubertal males had access to females. This seems likely as adult males showed higher rates of mounting and intromissions than did pubertal males (Dixson & Nevison, 1997). However, this difference does not appear to reflect differences in pubertal males ' sexual interest as they visually inspected and sniffed female genitalia at levels comparable to those seen in adult males (Dixson & Nevison, 1997). Thus, pubertal males are sexually interested in females, but most do not have the opportunity to mate with females. Females showed fewer sexual solicitations and initiated proximity less to pubertal males in comparison to adult males, further supporting the idea that lower levels of mounting by pubertal males reflects female rather than male choice (Dixson & Nevison, 1997).

In contrast to the data from macaques, in male savanna baboons, first sexual consortship occurs at approximately eight years of age, two to three years after initial testicular increases are observed (Alberts & Altmann, 1995). Thus, the expression and development of sexual behavior in nonhuman primates occurs independently of gonadal hormone changes during puberty as macaques began displaying sexual behavior prior to

puberty onset (Alberts & Altmann, 1995; Herman et al., 2006; Nieuwenhuijsen et al., 1988). Despite the effects of maternal rank on puberty onset in male savanna baboons, maternal rank did not influence age at first sexual consortship even after accounting for age when adult rank was attained (Alberts & Altmann, 1995). Thus, earlier puberty onset in male baboons with high-ranking mothers did not result in earlier reproductive success. The time between attaining adult rank and first sexual consortship was approximately 2.5 months, though the data ranged from 5-526 days and this was highly influenced by the number of cycling females in the group (Alberts & Altmann, 1995). First sexual consortship occurred after integration into the male social hierarchy, and therefore, it seems more likely that adult social rank would be a better predictor of age at first consortship, but further research is needed to evaluate this hypothesis.

Females

Females being mounted by males was first observed in female stumptail macaques within the first year of life (Nieuwenhuijsen et al., 1988), demonstrating that sexual receptivity, the willingness to be mounted, occurs well before puberty. First copulation occurred between one and three years of age, with first copulation with an adult male occurring between 2.5-3.5 years of age (Nieuwenhuijsen et al., 1988). Age at menarche is not known in this group of outdoor-socially-housed stumptail macaques, making it impossible to relate the expression of copulatory behavior to puberty onset. Rate of copulations increased about six months prior to first ovulation, which occurred at 3.4-4.2 years of age (Nieuwenhuijsen et al., 1988), possibly due to increasing levels of E2 that occur following puberty onset. Rate of copulation was not related to social rank, though social rank was related to the choice of copulation partners. Regardless of age,

high-ranking females, in comparison to middle- or low-ranking females, mated more frequently with high-ranking males (Nieuwenhuijsen et al., 1988). In female stumptail macaques, as in males, the expression of sexual behavior occurred well before puberty, but full copulation was temporally similar to the physiological changes during puberty.

In adult female rhesus macaques, social context alters the expression of sexual behavior in that social rank influences the coupling of gonadal hormones and the expression of sexual behavior (Wallen, 1990, 2001). High-ranking females will engage in consistently high levels of sexual behavior throughout the first half of the ovarian cycle - when E2 levels are low but increasing - demonstrating that sexual behavior is not tightly coupled to estradiol levels. In contrast to high-ranking females, low-ranking females show a tight coupling of behavior and E2 levels and only engage in sexual behavior around the time of the midcycle estradiol peak, when motivation is high enough to exceed any social consequences that occur as a result of mating. Thus, social rank alters the relationship between the expression of sexual behavior and gonadal hormones in adult females (Wallen, 1990, 2001).

In pubertal female rhesus macaques, female sexual initiation appeared to be influenced by social rank in that high-ranking females exhibited the highest rate of female sexual initiation at 2.5 years of age, the age at menarche, regardless of whether or not first ovulation occurred that season (Wallen & Zehr, 2004). In contrast to high-ranking females, middle-ranking females that ovulated at 2.5 years of age showed variability in the levels of sexual initiation observed. One middle-ranking female did not display any sexual initiation, whereas another female showed sexual interest, but levels of female sexual initiation were less than high-ranking females. None of the low-ranking

females ovulated or engaged in sexual behavior at 2.5 years of age (Wallen & Zehr, 2004; Zehr et al., 2005). Although the expression of female sexual initiation occurs around the time of puberty, this expression of behavior was not clearly related to the occurrence of menarche or first ovulation. Rather, the expression of sexual initiation appeared to be influenced by social rank, presumably with social rank influencing the level of estradiol required for female sexual initiation to occur. In females that ovulated at 2.5 years of age, there was an increase in female sexual initiation at 3.5 years of age in comparison to the levels of initiation observed a year earlier, when first ovulation occurred (Wallen & Zehr, 2004). At 3.5 years of age, high-ranking females displayed the highest level of sexual initiation and for longer time periods in comparison to middle- and low-ranking females. As in adult females, in pubertal females, middle- and low-ranking individuals displayed sexual initiation, but only around the time of peak E2 levels prior to ovulation (Wallen & Zehr, 2004).

It is possible that the effect of social rank on sexual behavior in pubertal females is a result of aggression from the male. Adult males were more likely to show aggression towards pubertal females and mounting seldom occurred (Wallen & Zehr, 2004) and thus, middle- and low-ranking females may not initiate sexual behavior because the male is unwilling. However, the effects of social rank on sexual behavior expression are similar for adult and pubertal females and therefore, it is not likely that the behavioral effects seen in pubertal females are solely the result of male aggression. It is more likely that social rank modifies sexual behavior by altering the importance of the relationship between estradiol and the expression of sexual behavior. For example, low-ranking females will only engage in sexual behavior for a few days when E2 is high and thus,

sexual motivation is high, whereas high-ranking females will engage in sexual behavior throughout the follicular phase, when E2 is low but increasing (Wallen, 1990, 2001). Although the expression of sexual behavior and neuroendocrine puberty are independent processes in female rhesus macaques, the effects of social rank on behavior and motivation likely result in changes in sexual behavior expression at the time of puberty.

In young peripubertal rhesus macaque females, mean age 3.8 years of age, E2 levels were significantly higher 10-30 days prior to the first ovulation of that season in comparison to adult females, 6 years of age or older (Wilson et al., 1982). However, E2 levels did not differ between peripubertal and adult females for the ten days leading up to the first E2 peak of the season. Thus, the longer exposure to greater E2 levels in younger females did not influence E2 levels leading up to the E2 peak or the magnitude of the E2 peak. These younger females also showed a longer period of copulatory activity in comparison to adult females, which is likely a result of the longer increase in E2 levels as this difference in behavior was restricted to the follicular phase of the menstrual cycle. Despite the longer period of observed sexual behavior in young females, there was no difference in the rate of copulatory behavior or births as a function of age. Thus, age only impacted the length of the copulatory period and these age differences in copulatory behavior were not a result of differences in social rank (Wilson et al., 1982).

In summary, the expression of sexual behavior appears to be independent of specific pubertal events in both males and females. Mate choice as well as distinct behavioral patterns likely alter the expression of sexual behavior observed around puberty. Social rank does not influence sexual behavior prior to puberty, but influences behavior during puberty in both males and females. The effects of social rank on sexual

behavior during puberty are likely a result of access to mates, though differences in sexual behavior as a result of rank cannot be discounted.

Conclusion

Puberty reflects an integration of the hormonal and social history of an individual and is modulated by the current environmental and social context. Thus, there are many points during development when events later affect the timing of puberty onset or the speed with which pubertal changes occur. The timing of puberty is also one of the more consistent sex differences, with females on average showing earlier puberty onset than do males. Interestingly, the neuroendocrine mechanisms involved in the timing of puberty appear to be sensitive to androgens (both prenatal and neonatal) in males, but do not appear to be sensitive in females. In contrast, leptin appears to influence pubertal events in females, but not in males. Why there would be this asymmetry in response is unclear.

The dramatic changes in sexual behavior that occur around puberty in both males and females raise the possibility that the pubertal elevation in gonadal hormones organizes adult sexual response patterns (Schulz et al., 2009), the result of which is that behavioral patterns that were previously expressed independently of the animal's hormonal state now come under the control of the activational effects of gonadal steroids. For example, male rhesus monkeys routinely display mounting behavior as juveniles when their testes are nonfunctional. However, after puberty, castration or suppression of testicular function reduces mounting, which is only restored when T levels are increased (Wallen, et al., 1991, Chambers and Phoenix, 1983), even though androgens are not necessary for the display of the mounting motor pattern (Wallen, 1990). Social rank appears to influence sexual behavior in males and females, beginning at the time of

puberty (Herman et al., 2006; Wallen & Zehr, 2004). Possibly what is organized at puberty is hormonal modulation of sexual behavior such that it is displayed in relation to the social context and coordinated such that it increases reproductive success."

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References

Abbott, D. H., & Hearn, J. P. (1978). Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, *Callithrix jacchus*. *J Reprod Fertil, 53,* 155-166.

Abbott, D. H., Hodges, J. K., & George, L. M. (1988). Social status controls LH secretion and ovulation in female marmoset monkeys (*Callithrix jacchus*). *J Endocrinol, 117,* 329-339.

Abbott, D. H., McNeilly, A. S., Lunn, S. F., Hulme, M. J., & Burden, F. J. (1981). Inhibition of ovarian function in subordinate female marmoset monkeys (*Callithrix jacchus jacchus*). *J Reprod Fertil, 63,* 335-345.

Abbott, D. H., Saltzman, W., Schultz-Darken, N. J., & Smith, T. E. (1997). Specific neuroendocrine mechanisms not involving generalized stress mediate social regulation of female reproduction in cooperatively breeding marmoset monkeys. *Ann N Y Acad Sci, 807,* 219–238.

Ahima, R. S., Dushay, J., Flier, S. N., Prabakaran, D., & Flier, J. S. (1997). Leptin accelerates the onset of puberty in normal female mice. *J Clin Invest, 99,* 391-395.

Akhtar, F. B., Marshall, G. R., Wickings, E. J., & Nieschlag, E. (1983). Reversible induction of azoospermia in rhesus monkeys by constant infusion of a gonadotropin-releasing hormone agonist using osmotic minipumps. *J Clin Endocrinol Metab, 56,* 534-540.

Alberts, S. C., & Altmann, J. (1995). Preparation and activation: determinants of age at reproductive maturity in male baboons. *Behav Ecol Sociobiol, 36,* 397-406.

Bales, K. L., French, J. A., McWilliams, J., Lake, R. A., & Dietz, J. M. (2006). Effects of social status, age, and season on androgen and cortisol levels in wild male golden lion tamarins (*Leontopithecus rosalia*). *Horm Behav, 49*, 88-95.

Barker-Gibb, M. L., Sahu, A., Pohl, C. R., & Plant, T. M. (2002). Elevating circulating leptin in prepubertal male rhesus monkeys (*Macaca mulatta*) does not elicit precocious gonadotropin-releasing hormone release, assessed indirectly. *J Clin Endocrinol Metab, 87*, 4976-4983.

Barrett, J., Abbott, D. H., & George, L. M. (1990). Extension of reproductive suppression by pheromonal cues in subordinate female marmoset monkeys, *Callithrix jacchus*. *J Reprod Fertil, 90*, 411-418.

Belsky, J., Steinberg, L. D., Houts, R. M., Friedman, S. L., DeHart, G., Cauffman, E., Roisman, G. I., Halpern-Felsher, B. L., Susman, E., & NICHD Early Child Care Research Network. (2007). Family rearing antecedents of pubertal timing. *Child Dev, 78*(4), 1302-1321.

Bercovitch, F. B. (1993). Dominance rank and reproductive maturation in male rhesus macaques (*Macaca mulatta*). *J Reprod Fertil, 99*, 113-120.

Bercovitch, F. B., & Strum, S. C. (1993). Dominance rank, resource availability, and reproductive maturation in female savanna baboons. *Behav Ecol Sociobiol, 33*, 313-318.

Birnie, A. K., Smith, A. S., Nali, C., & French, J. A. (2011). Social and developmental influences on urinary androgen levels in young male white-faced marmosets (*Callithrix geoffroyi*). *Am J Primatol, 73*, 378-385.

Bogaert, A. F. (2005). Age at puberty and father absence in a national probability sample.

J Adolesc, *28*, 541-546.

Chambers, K. C., & Phoenix, C. H. (1983). Sexual behavior in response to testosterone in old long-term-castrated rhesus males. *Neurobiol Aging*, *4*, 223-227.

Charpentier, M. J. E., Tung, J., Altmann, J., & Alberts, C. (2008). Age at maturity in wild baboons: Genetic, environmental and demographic influences. *Mol Ecol*, *17*, 2026-2040.

Chehab, F. F., Lim, M. E., Lu, R. (1996). Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nat Genet*, *12*, 318-320.

Chik, C. L., Almeida, O. F. X., Libré, E. A., Booth, J. D., Renquist, D., & Merriam, G. R. (1992). Photoperiod-driven changes in reproductive function in male rhesus monkeys. *J Clin Endocrinol Metab*, *74*, 1068-1074.

Chongthammakun, S., & Terasawa, E. (1993). Negative feedback effects of estrogen on luteinizing hormone-releasing hormone release occur in pubertal, but not prepubertal, ovariectomized female rhesus monkeys. *Endocrinology*, *132*, 735-743.

Dixson, A. F., & Nevison, C. M. (1997). The socioendocrinology of adolescent development in male rhesus monkeys (*Macaca mulatta*). *Horm Behav*, *31*, 126-135.

Frisch, R.E. (1972). Weight at menarche: similarity for well-nourished and undernourished girls at differing ages, and evidence for historical constancy. *Pediatrics*, *50*, 445–450.

Frisch, R.E., & McArthur, J.W. (1974). Menstrual cycles: fatness as a determinant of

minimum weight for height necessary for their maintenance or onset. *Science, 185*, 949–951.

Gay, V. L., & Plant, T. M. (1987). N-methyl-D,L-aspartate elicits hypothalamic gonadotropin-releasing hormone release in prepubertal male rhesus monkeys (*Macaca mulatta*). *Endocrinology, 120*, 2289-2296.

Gay, V. L., & Plant, T. M. (1988). Sustained intermittent release of gonadotropin-releasing hormone in the prepubertal male rhesus monkey induced by N-methyl-DL-aspartic acid. *Neuroendocrinology, 48*, 147-152.

Ginther, A. J., Carlson, A. A., Ziegler, T. E., & Snowdon, C. T. (2002). Neonatal and pubertal development in males of a cooperatively breeding primate, the cotton-top tamarin (*Saguinus oedipus oedipus*). *Biol Reprod, 66*, 282-290.

Ginther, A. J., Ziegler, T. E., & Snowdon, C. T. (2001). Reproductive biology of captive male cottontop tamarin monkeys as a function of social environment. *Anim Behav, 61*, 65-78.

Goy, R. W., Bercovitch, F. B., & McBrair, M. C. (1988). Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. *Horm Behav, 22*, 552-571.

Graber, J. A., Brooks-Gunn, J., & Warren, M. P. (1995). The antecedents of menarcheal age: Heredity, family environment, and stressful life events. *Child Dev, 66*, 346-359.

Grumbach, M. M., & Styne, D. M. (2003). Puberty: Ontogeny, neuroendocrinology, physiology and disorders. In P. R. Larsen, H. M. Kronenberg, S. M. Melmed, & K. S. Polonsky (Eds.), *Williams textbook of endocrinology* (10th ed., pp. 1115-

1286). Philadelphia: Saunders.

Guerriero, K. A., Keen, K. L., Millar, R. P., & Terasawa, E. (2012). Developmental changes in GnRH release in response to kisspeptin agonist and antagonist in female rhesus monkeys (*Macaca mulatta*): Implication for the mechanism of puberty. *Endocrinology, 153,* 825-836.

Heber, D., Bhasin, S., Steiner, B., & Swerdloff, R. S. (1984). The stimulatory and down-regulatory effects of a gonadotropin-releasing hormone agonist in man. *J Clin Endocrinol Metab, 58,* 1084-1088.

Herman, R. A., Jones, B., Mann, D. R., & Wallen, K. (2000). Timing of prenatal androgen exposure: Anatomical and endocrine effects on juvenile male and female rhesus monkeys. *Horm Behav, 38,* 52-66.

Herman, R. A., Zehr, J. L., & Wallen, K. (2006). Prenatal androgen blockade accelerates pubertal development in male rhesus monkeys. *Psychoneuroendocrinology, 31,* 118-130.

Hoff, J. D., Quigley, M. E., & Yen, S. S. C. (1983). Hormonal dynamics at midcycle: A reevaluation. *J Clin Endocrinol Metab, 57,* 792-796.

Horlick, M. B., Rosenbaum, R. V., Nicolson, M., Levine, L. S., Fedun, B., Wang, J., Pierson, R. N. Jr., & Leibel, R. L. (2000). Effect of puberty on the relationship between circulating leptin and body composition. *J Clin Endocrinol Metab, 85,* 2509-2518.

Keen, K. L., Burich, A. J., Mitsushima, D., Kasuya, E., & Terasawa, E. (1999). Effects of pulsatile infusion of the $GABA_A$ receptor blocker bicuculline on the onset of puberty in female rhesus monkeys. *Endocrinology, 140,* 5257-5266.

Keen, K. L., Wegner, F. H., Bloom, S. R. Ghatei, M. A., & Terasawa, Ei. (2008). An increase in kisspeptin-54 release occurs with the pubertal increase in luteinizing hormone-releasing hormone-1 release in the stalk-median eminence of female rhesus monkeys *in Vivo*. *Endocrinology*, *149*, 4151-4157.

Knobil, E. (1974). On the control of gonadotropin secretion in rhesus monkeys. *Recent Prog Horm Res*, *30*, 1-46.

Knobil, E. (1980). The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res*, *36*, 53-88.

Koford, C. B. (1963). Rank of mothers and sons in bands of rhesus monkeys. *Science*, *141*, 356-357.

Lebrethon, M. C., Vandermissen, E., Gerard, A., Parent, A. S., Junien, J. L., & Bourguignon, J. P. (2000). In vitro stimulation of the pre-pubertal rat gonadotropin-releasing hormone pulse generator by leptin and neuropeptide Y through distinct mechanisms. *Endocrinology*, *141*, 1464-1469.

Mahachoklertwattana, P., Sanchez, J., Kaplan, S. L., & Grumbach, M. M. (1994). N-methyl-D-aspartate (NMDA) receptors mediate the release of gonadotropin-releasing hormone (GnRH) by NMDA in a hypothalamic GnRH neuronal cell line (GT1-1). *Endocrinology*, *134*, 1023-1030.

Mann, D. R., Akinbami, M. A., Gould, K. G., & Castracane, V. D. (2000). A longitudinal study of leptin during development in the male rhesus monkey: The effect of body composition and season on circulating leptin levels. *Biol Reprod*, *62*, 285-291.

Mann, D. R., Akinbami, M. A., Gould, K. G., & Castracane, V. D. (2002). Leptin and thyroxine during sexual development in male monkeys: Effect of neonatal

gonadotropin-releasing hormone antagonist treatment and delayed puberty on the developmental pattern of leptin and thyroxine secretion. *Eur J Endocrinol, 146,* 891-898.

Mann, D. R., Akinbami, M. A., Gould, K. G., Paul, K., & Wallen, K. (1998). Sexual maturation in male rhesus monkeys: Importance of neonatal testosterone exposure and social rank. *J Endocrinol, 156,* 493-501.

Mann, D. R., Akinbami, M. A., Gould, K. G., Tanner, J. M., & Wallen, K. (1993). Neonatal treatment of male monkeys with a gonadotropin-releasing hormone agonist alters differentiation of central nervous system centers that regulate sexual and skeletal development. *J Clin Endocrinol Metab, 76,* 1319-1324.

Mann, D. R., Ansari, A. A., Akinbami, M. A., Wallen, K., Gould, K. G., & McClure, H. M. (1994). Neonatal treatment with luteinizing hormone-releasing hormone analogs alters peripheral lymphocyte subsets and cellular and humorally mediated immune responses in juvenile and adult male monkeys. *J Clin Endocrinol Metab, 78,* 292-298.

Mann, D. R., Davis-DaSilva, M., Wallen, K., Coan, P., Evans, D. E., & Collins, D. C. (1984). Blockade of neonatal activation of the pituitary–testicular axis with continuous administration of a gonadotropin-releasing hormone agonist in male rhesus monkeys. *J Clin Endocrinol Metab, 58,* 262–267.

Mann, D. R., Gould, K. G., Collins, D. C., & Wallen, K. (1989). Blockade of neonatal activation of the pituitary-testicular axis: Effect on peripubertal luteinizing hormone and testosterone secretion and on testicular development in male monkeys. *J Clin Endocrinol Metab, 68,* 600-607.

Mann, D. R., Lunn, S. F., Akinbami, M. A., Samuel, K., Waterfall, M., & Fraser, H. M. (1999). Effect of neonatal treatment with a GnRH antagonist on development of the cell-mediated immune response in marmosets. *Am J Reprod Immunol, 42*, 175-186.

Marshall, W. A., & Tanner, J. M. (1969). Variations in pattern of pubertal changes in girls. *Arch Dis Child, 44*, 291-303.

Marshall, W. A., & Tanner, J. M. (1970). Variations in the pattern of pubertal changes in boys. *Arch Dis Child, 45*, 13-23.

Matkovic, V., Ilich, J. Z., Skugor, M., Badenhop, N. E., Goel, P., Clairmont, A., Klisovic, D., Nahhas, R. W., & Landoll, J. D. (1997). Leptin is inversely related to age at menarche in human females. *J Clin Endocrinol Metab, 82*(10), 3239-3245.

Mitsushima, D., Hei, D. L., & Terasawa, E. (1994). γ-Aminobutyric acid is an inhibitory neurotransmitter restricting the release of luteinizing hormone-releasing hormone before the onset of puberty. *Proc Natl Acad Sci U S A, 91*, 395-399.

Moffit, T. E., Caspi, A., Belsky, J., & Silva, P. A. (1992). Childhood experience and the onset of menarche: A test of a sociobiological model. *Child Dev, 63*, 47-58.

Molskness, T. A., Woodruff, T. K., Hess, D. L., Dahl, K. D., & Stouffer, R. L. (1996). Recombinant human inhibin-A administered early in the menstrual cycle alters concurrent pituitary and follicular, plus subsequent luteal, function in rhesus monkeys. *J Clin Endocrinol Metab, 81*, 4002-4006.

Nakai, Y., Plant, T. M., Hess, D. L., Keogh, E. J., & Knobil, E. (1978). On the sites of the negative and positive feedback actions of estradiol in the control of gonadotropin secretion in the rhesus monkey. *Endocrinology, 102*, 1008-1014.

Nieuwenhuijsen, K., Bonke-Jansen, M., Broekhuijzen, E., De Neef, K. J., Van Hooff, J. A. R. A. M., Van Der Werff Ten Bosch, J. J., & Slob, A. K. (1988). Behavioral aspects of puberty in group-living stumptail monkeys (*Macaca arctoides*). *Physiol Behav*, *42*, 255-264.

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T., & Collins, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science*, *269*, 540-543.

Phoenix, C. H., Slob, A. K., & Goy, R. W. (1973). Effects of castration and replacement therapy on sexual behavior of adult male rhesuses. *J Comp Physiol Psychol*, *84*, 472-481.

Plant, T. M. (2001). Neurobiological bases underlying the control of the onset of puberty in the Rhesus Monkey: A representative higher primate. *Front Neuroendocrinol*, *22*, 107-139.

Plant, T. M., & Dubey, A. K. (1984). Evidence from the Rhesus Monkey (*Macaca mulatta*) for the view that negative feedback control of luteinizing hormone secretion by the testis is mediated by a deceleration of hypothalamic gonadotropin-releasing hormone pulse frequency. *Endocrinology*, *115*, 2145-2153.

Plant, T. M., Gay, V. L., Marshall, G. R., & Arsian, M. (1989). Puberty in monkeys is triggered by chemical stimulation of the hypothalamus. *Proc Natl Acad Sci U S A*, *86*, 2506-2510.

Plant, T. M., Krey, L. C., Moossy, J., McCormack, J. T., Hess, D. L., & Knobil, E. (1978). The arcuate nucleus and the control of gonadotropin and prolactin

secretion in the female rhesus monkey (Macaca mulatta). *Endocrinology*, *102*, 52-62.

Ramaswamy, S., Marshall, G. R., McNeilly, A. S., & Plant, T. M. (2000). Dynamics of the follicle-stimulating hormone (FSH)-Inhibin B feedback loop and its role in regulating spermatogenesis in the adult male rhesus monkey (*Macaca mulatta*) as revealed by unilateral orchidectomy. *Endocrinology, 141*, 18-27.

Ramaswamy, S., & Plant, T. M. (2001). Operation of the follicle-stimulating hormone (FSH)-inhibin B feedback loop in the control of primate spermatogenesis. *Mol Cell Endocrinol*, *180*, 93-101.

Rowe, D. C. (2000). Environmental and genetic influences on pubertal development: Evolutionary life history traits? In J. L. Rodgers, D. C. Rowe, & W. B. Miller (Eds.), *Genetic influences on human fertility and sexuality: Theoretical and empirical contributions from the biological and behavioral sciences* (pp. 147-168). Boston: Kluwer, Academic Publishers.

Rutters, F., Nieuwenhuizen, A. G., Verhoef, S. P. M., Lemmens, S. G. T., Vogels, N., & Westerterp-Plantenga, M. S. (2009). The relationship between leptin, gonadotropic hormones, and body composition during puberty in a Dutch children cohort. *Eur J Endocrinol*, *160*, 973-978.

Saltzman, W., Pick, R. R., Salper, O. J., Liedl, K. J., & Abbott, D. H. (2004). Onset of plural cooperative breedingin common marmoset families following replacement of the breeding male. *Anim Behav*, *68*, 59-73.

Saltzman, W., Schultz-Darken, N. J., Severin, J. M., & Abbott, D. H. (1997). Escape from social suppression of sexual behavior and of ovulation female common

marmosets. *Ann N Y Acad Sci, 807,* 567-570.

Schneider, J. E., Klingerman, C. M., & Abdulhay, A. (2012). Sense and nonsense in metabolic control of reproduction. *Front Endocrinol, 3*:26, doi: 10.3389/fendo.2012.00026.

Schwartz, S. M., Wilson, M. E., Walker, M. L., & Collins, D. C. (1988). Dietary influences of growth and sexual maturation in premenarchial rhesus monkeys. *Horm Behav, 22,* 231-251.

Schulz, K.M., Molenda-Figueira, H.A., Sisk, C.L. (2009). Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. *Horm Behav* 55, 597-604.

Semiz, S., Kurt, F., Kurt, D. T., Zencir, M., & Sevinc, O. (2009). Factors affecting onset of puberty in Denizli province in Turkey. *Turk J Pediatr, 51,* 49-55.

Setchell, J. M., Wickings, E. J., & Knapp, L. A. (2006). Life history in male mandrills (*Mandrillus sphinx*): Physical development, dominance rank, and group association. *Am J Phys Anthropol, 131,* 498-510.

Shahab, M., Mastronardi, C., Seminara, S. B., Crowley, W. F., Ojeda, S. R., & Plant, T. (2005). Increased hypothalamic GPR54 signaling: A potential mechanism for initiation of puberty in primates. *Proc Natl Acad Sci U S A, 102,* 2129-2134.

Smith, T. E., Schaffner, C. M., & French, J. A. (1997). Social and developmental influences on reproductive function in female Wied's black tufted-ear marmosets (*Callithrix kuhli*). *Horm Behav, 31,* 159-168.

Sousa, M. B. C., Albuquerque, A. C. S. D. R., Albuquerque, F. D. S., Araujo, A., Yamamoto, M. E., & Arruda, M. D. F. (2005). Behavioral strategies and

hormonal profiles of dominant and subordinate common marmosets (*Callithrix jacchus*) females in wild monogamous groups. *Am J Primatol, 67*, 37-50.

Suter, K. J., Pohl, C. R., & Plant, T. M. (1998). The pattern and tempo of the pubertal reagumentation of open-loop pulsatile gonadotropin-relasing hormone release assessed indirectly in the male rhesus monkey (*Macaca mulatta*). *Endocrinology, 139*, 2774-2783.

Terasawa, E., Krook, C., Eman, S., Watanabe, G., Bridson, W. E., Sholl, S. A., & Hei, D. L. (1987). Pulsatile luteinizing hormone (LH) release during the progesterone-induced LH surge in the female rhesus monkey. *Endocrinology, 120*, 2265-2271.

Terasawa, E., Kurian, J. R., Keen, K. L., Shiel, N. A., Colman, R. J., & Capuano, S. V. (2012). Body weight impact on puberty: Effects of high-calorie diet on puberty onset in female rhesus monkeys. *Endocrinology, 153*, 1696-1705.

Terasawa, E., Rodriguez-Sierra, J. F., Dierschke, D. J., Bridson, W. E., & Goy, R. W. (1980). Positive feedback effect of progesterone on luteinizing hormone (LH) release in cyclic female rhesus monkeys: LH response occurs in two phases. *J Clin Endocrinol Metab, 51*, 1245-1250.

Tither, J. M., & Ellis, B. J. (2008). Impact of fathers on daughters' age at menarche: A genetically and environmentally controlled sibling study. *Dev Psychol, 44*, 1409-1420.

Wallen, K. (1990). Desire and ability: hormones and the regulation of female sexual behavior. *Neurosci Biobehav Rev, 14*, 233-241.

Wallen, K. (1996). Nature needs nurture: the interaction of hormonal and social influences on the development of behavioral sex differences in rhesus monkeys.

Horm Behav, *30*, 364-378.

Wallen, K. (2001). Sex and context: Hormones and primate sexual motivation. *Horm Behav*, *40*, 339-357.

Wallen, K. (2005). Hormonal influences on sexually differentiated behavior in nonhuman primates. *Front Neuroendocrinol*, *26*, 7-26.

Wallen, K., Eisler, J. A., Tannenbaum, P. L., Nagell, K. M., & Mann, D. R. (1991). Antide (NAL-LYS GnRH antagonist) suppression of pituitary-testicular function and sexual behavior in group-living rhesus monkeys. *Physiol Behav*, *50*, 429-435.

Wallen, K., & Zehr, J. L. (2004). Hormones and history: The evolution and development of primate female sexuality. *J Sex Res*, *41*, 101-112.

Watanabe, G., & Terasawa, E. (1989). In vivo release of luteinizing hormone releasing hormone increases with puberty in the female rhesus monkey. *Endocrinology*, *125*, 92-99.

Wierson, M., Long, P. J., & Forehand, R. L. (1993). Toward a new understanding of early menarche: The role of environmental stress in pubertal timing. *Adolescence*, *28*, 913-924.

Wildt, L., Marshall, G., & Knobil, E. (1980). Experimental induction of puberty in the infantile female rhesus monkey. *Science*, *207*, 1373-1375.

Wilen, R., & Naftolin, F. (1976). Age, weight, and weight gain in the individual pubertal female rhesus monkey (*Macaca mulatta*). *Biol Reprod*, *15*, 356-360.

Wilson, M. E., Bounar, S., Godfrey, J., Michopoulos, V., Higgins, M., & Sanchez, M. (2013). Social and emotional predictors of the tempo of puberty in female rhesus monkeys. *Psychoneuroendocrinology*, *38*, 67-83.

Wilson, M. E., Fisher, J., Chikazawa, K., Yoda, R., Legendre, A., Mook, D., & Gould, K. G. (2003). Leptin administration increases nocturnal concentrations of luteinizing hormone and growth hormone in juvenile female rhesus monkeys. *J Clin Endocrinol Metab, 88,* 4874-4883.

Wilson, M. E., & Gordon, T. P. (1989a). Season determines timing of first ovulation in rhesus monkeys (*Macaca mulatta*) housed outdoors. *J Reprod Fertil, 86,* 435-444.

Wilson, M. E., & Gordon, T. P. (1989b). Short-day melatonin pattern advances puberty in seasonally breeding rhesus monkeys (Macaca mulatta). *J Reprod Fertil, 85,* 583-591.

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. *J Reprod Fertil, 70,* 625-633.

Wilson, M. E., Gordon, T. P., & Collins, D. C. (1982). Age differences in copulatory behavior and serum 17β-Estradiol in female rhesus monkeys. *Physiol Behav, 28,* 733-737.

Wilson, M. E., Gordon, T. P., & Collins, D. C. (1986). Ontogeny of luteinizing hormone secretion and first ovulation in seasonal breeding rhesus monkeys. *Endocrinology, 118,* 293-301.

Wilson, M. E., Gordon, T. P., Rudman, C. G., & Tanner, J. M. (1988). Effects of a natural versus artificial environment on the tempo of maturation in female rhesus monkeys. *Endocrinology, 123,* 2653-2661.

Wilson, M. E., & Kinkead, B. (2008). Gene-environment interactions, not neonatal growth hormone deficiency, time puberty in female rhesus monkeys. *Biol Reprod,*

78, 736-743.

Xia, L., Van Vugt, D., Alston, E. J., Luckhaus, J., & Ferin, M. (1992). A surge of gonadotropin-releasing hormone accompanies the estradiol-induced gonadotropin surge in the rhesus monkey. *Endocrinology*, *131*, 2812-2820.

Yu, W. H., Walczewska, A., Karanth, S., & McCann, S. M. (1997). Nitric oxide mediates leptin-induced luteinizing hormone-releasing hormone (LHRH) and LHRH and leptin-induced LH release from the pituitary gland. *Endocrinology*, *138*, 5055-5058.

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. *Biol Reprod*, *72*, 1087-1094.

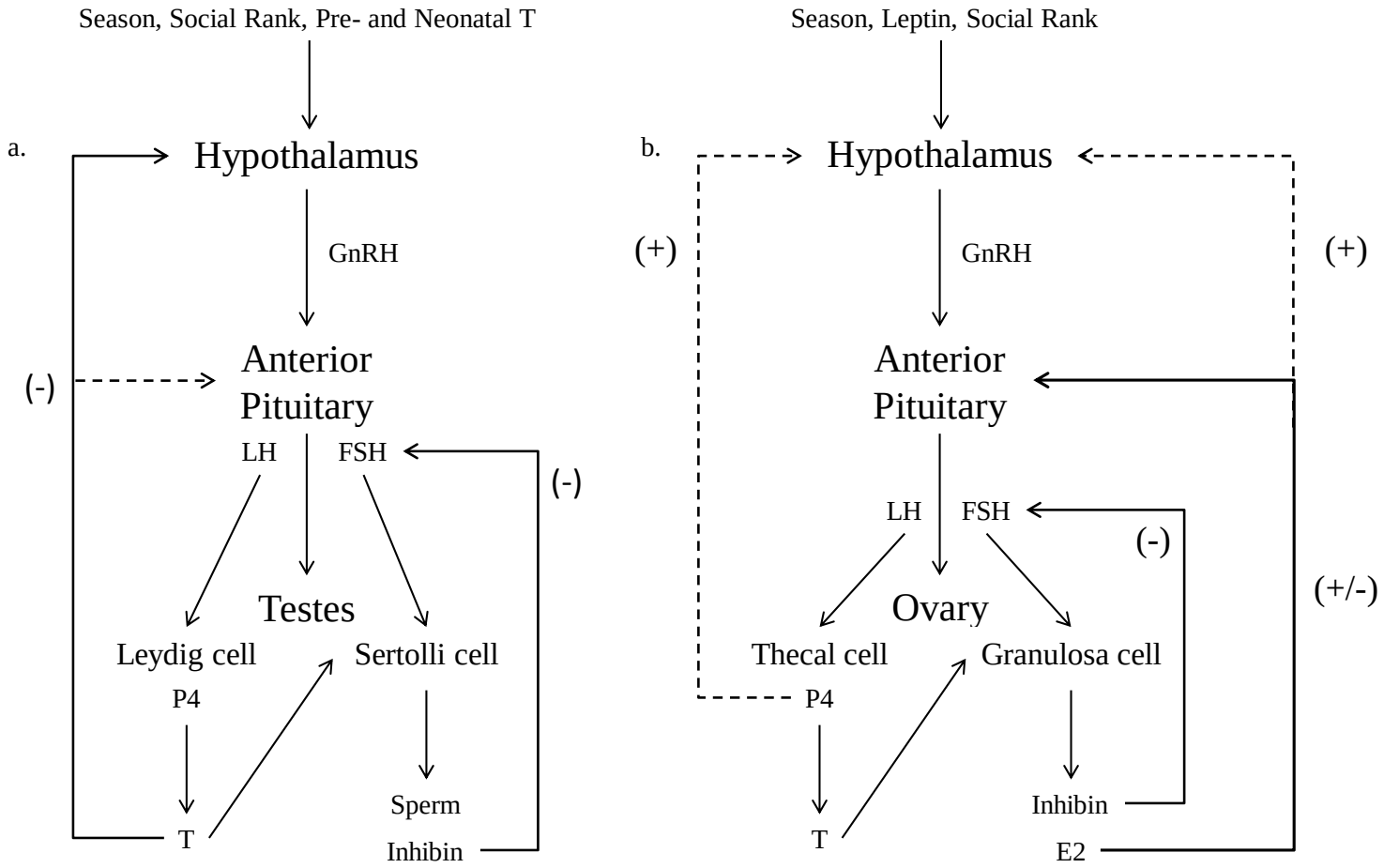
Ziegler, T. E., Savage, A., Scheffler, G., & Snowdon, C. T. (1987). The endocrinology of puberty and reproductive functioning in female cotton-top tamarins (*Saguinus oedipus*) under varying social conditions. *Biol Reprod*, *37*, 618-627.

Figure Captions

Figure 1. Primary (solid line) and secondary (dashed line) mechanisms of the hypothalamic-pituitary-gonadal axis feedback loop in (a) male and (b) female nonhuman primates.

Figure 2. The rate of double-foot clasp mounting by male rhesus macaques during their juvenile and peripubertal time in relation to the sex of the partner. Males mount male and female partners almost equally as juveniles, but markedly increase their mounting of females peripubertally (Adapted from Wallen, 2001).

Figure 3. The rate of double-foot clasp mounting by male rhesus macaques during their juvenile and peripubertal time in relation to the sex of the partner and whether they were observed to show the ejaculatory reflex during the peripubertal time. Males did not display the ejaculatory reflex as juveniles, but are categorized based on their peripubertal behavior. Only males who had been observed to ejaculate with a female showed a marked increase in mounting of females (Adapted from Wallen, 2001).


a.
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b.
Season, Leptin, Social Rank
Hypothalamus
(+)
GnRH
(+)
Anterior Pituitary
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FSH
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Granulosa cell
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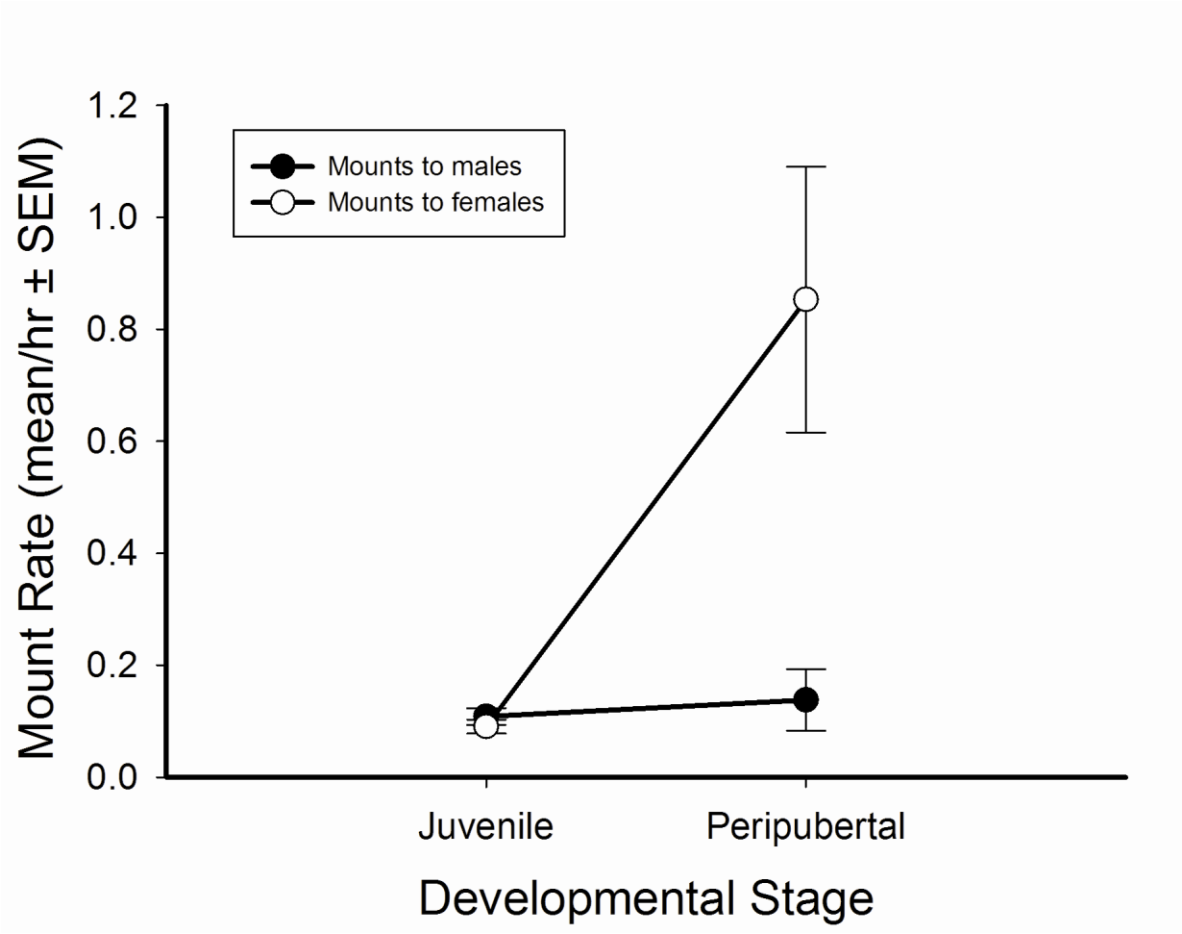
Figure 1.


Mount Rate (mean/hr ± SEM)
1.2
1.0
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0.2
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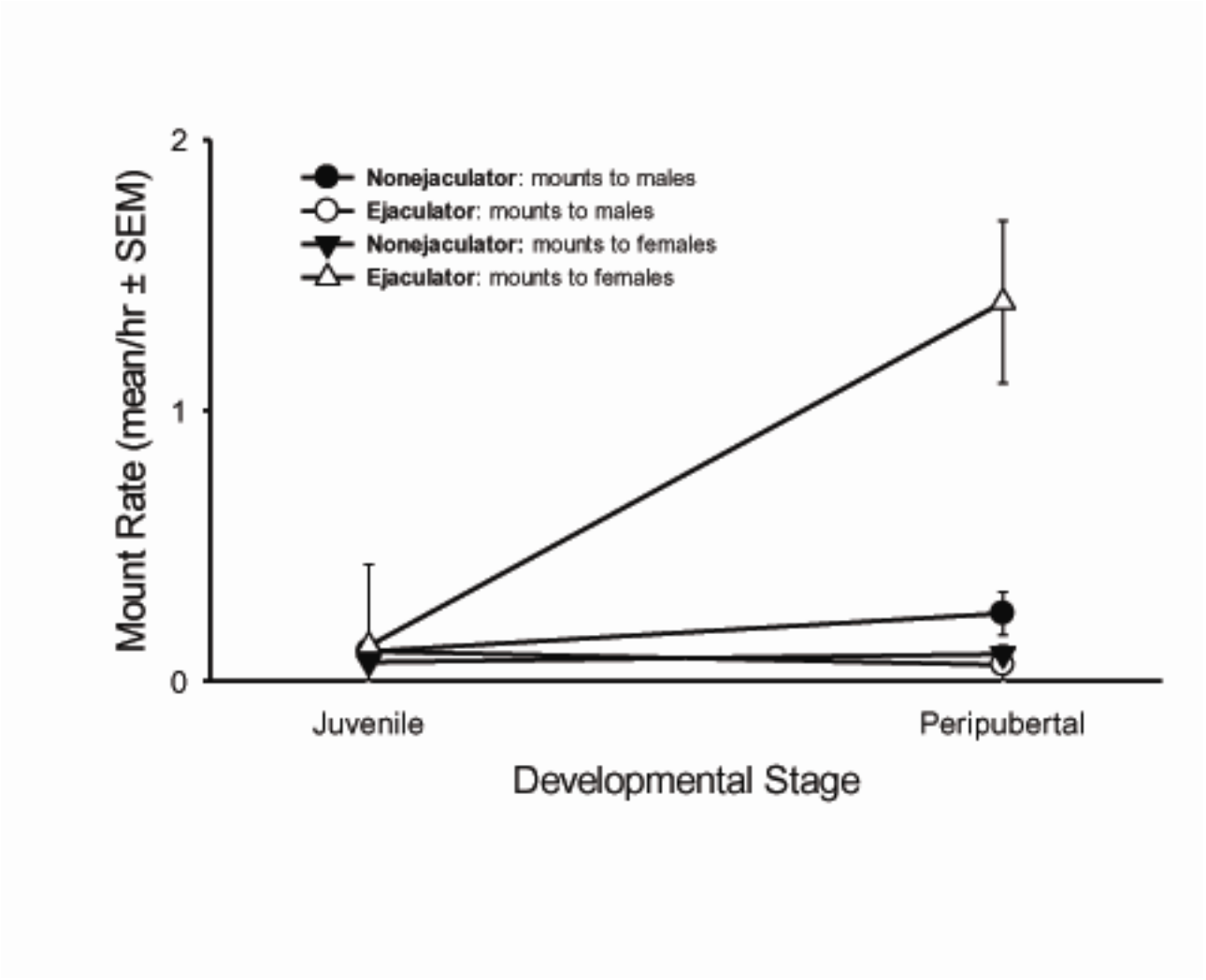
Mounts to males
Mounts to females
Juvenile
Peripubertal
Developmental Stage

Figure 2.


Mount Rate (mean/hr ± SEM)
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Nonejaculator: mounts to males
Ejaculator: mounts to males
Nonejaculator: mounts to females
Ejaculator: mounts to females
Juvenile
Peripubertal
Developmental Stage

Figure 3.

Running Head: Neonatal Amygdala Lesions and Puberty

Neonatal Amygdala Lesions Advance Pubertal Timing in Female Rhesus Macaques

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Abstract

Puberty is initiated by the cyclic release of gonadotropin-releasing hormone (GnRH) from the hypothalamus and culminates in adult reproductive function. Despite the trend for earlier menarche in humans and rhesus macaques, there exists a considerable variation in the timing of puberty. Social context influences the timing of puberty in both humans and nonhuman primates, such as delayed first ovulation in low-ranking rhesus macaques, but the brain region(s) mediating the effects of social context on pubertal timing are unknown. The amygdala is important for responding to social information and thus, is a potential brain region mediating the effects of social context on pubertal timing. In this study, female rhesus macaques living in social groups received bilateral neurotoxic amygdala lesions, that produced an average of 77% damage, at approximately one month of age and starting at 14 months of age, pubertal timing was examined. Neonatal lesions affected pubertal timing with lesioned females experiencing significantly earlier menarche (530.86±20.13 days) than did control females (734.44±61.56 days). Body weight at 1.5 years of age was unrelated to age at menarche in lesioned females ($r(7) = .27$, $p = .565$), though there was a nonsignificant statistical trend in control females ($r(9) = .66$, $p = .051$). By contrast, body weight of lesioned females at 1.5yr predicted age at first ovulation in lesioned females ($\beta = .93$, $t(3) = 4.43$, $p = .021$; $R^2 = .87$, $F(1,4) = 19.59$, $p = .021$), but not in controls ($\beta = -.59$, $t(7) = -1.92$, $p = .097$; $R^2 = .34$, $F(1,8) = 3.68$, $p = .097$). Social rank of lesioned females was related to age at menarche ($r(7) = .82$, $p = .023$), but not first ovulation, but was not related to either event in control females. It seems unlikely that earlier puberty in lesioned females reflects the removal of a social-context brake on pubertal timing. More likely is that the

effects of amygdalectomy on pubertal timing involve its modulation of GABAergic mechanisms intimately involved in pubertal timing.

Menarche, first menstruation, is one hallmark of the onset of puberty in humans (Marshall & Tanner, 1969), and indicates the onset of the transition to adult reproductive function of the hypothalamic-pituitary-gonadal (HPG) axis (as reviewed by Grumbach & Styne, 2003). In humans, menarche doesn't occur at a fixed developmental time, but is influenced by the environmental and social context (Moffit, Caspi, Belsky, & Silva, 1992; Wierson, Long, & Forehand, 1993; Graber, Brooks-Gunn, & Warren, 1995; Ersoy, Balkan, Gunay, & Egemen, 2005). Over the last 100 years, human menarche has been occurring increasingly earlier in development (Herman-Giddens, 2006; Morris, Jones, Schoemaker, Ashworth, & Swerdlow, 2011), for reasons that are unclear, but seem likely to reflect alterations in the nutritional or social environment. Within this secular trend towards earlier menarche is evidence that menarche varies between individuals as a result of epigenetic factors, such as the timing of a mother's menarche influencing her daughter's menarche (Ersoy et al., 2005; Graber et al., 2005), or changes in a girl's social environment, such as parental divorce (Wierson et al., 1993) or a new, unfamiliar man in the household (Graber et al., 1995) hastening menarche. It seems apparent that some system or systems responsible for monitoring the developing girl's physical and social environment and interact with the HPG axis to regulate the timing of puberty onset. However, the brain mechanisms monitoring the social environment and interacting with the HPG axis to time pubertal onset in response to specific social conditions are unknown.

Female rhesus macaques (*Macaca mulatta*), like humans, are menstrual primates with a developmental trajectory that, while faster than humans, shows similar reproductive milestones to those seen in girls and women (Marshall & Tanner, 1969;

Foster, 1977; Resko, Goy, Robinson, & Norman, 1982). In rhesus macaques, as in humans, menarche is one marker of the onset of the pubertal cascade of neuroendocrine changes that will lead to adult reproductive competency (Foster, 1977; Resko et al., 1982). As in humans, social context affects pubertal timing in female rhesus macaques. Higher-ranking female rhesus macaques had an earlier age at first sexual swelling or menarche than did lower-ranking females (Wilson, Bounar, Godfrey, Michopoulos, Higgins, & Sanchez, 2013), but this relationship between rank and menarche is not consistently found as other data showed no relationship between rank and menarche (Zehr, Van Meter, & Wallen, 2005; Wilson & Kinkead, 2008). Although it is not clear whether social rank influences age at menarche, there is clear evidence that the interval from menarche to full reproductive function is shorter in females with higher social rank than it is in subordinate female monkeys (Zehr, et al., 2005; Wilson et al., 2013). Thus, the social context modulates the activation and expression of HPG axis function, but as in humans, the neural modulatory mechanism(s) are unknown.

A prime candidate for the transduction of social contextual information is the amygdaloid complex, which integrates social contextual information (Rosvold, Mirsky, & Pribram, 1954; Thompson, Schwartzbaum, & Harlow, 1969; Kling & Cornell, 1971; Petrulis & Johnson, 1999) and has robust connections to the HPG axis (as reviewed by Amaral, Price, Pitkänen, & Carmichael, 1992). In female rats, lesions of the amygdala alter the timing of pubertal onset with the specific effect varying with the developmental timing of the lesion. Bilateral lesions of the anterior medial amygdala at 15 days of age resulted in delayed pubertal onset in female rats, whereas lesions at 21 days of age resulted in earlier pubertal onset, and lesions at 26 days of age did not alter pubertal onset

(Döcke, 1974; Döcke, Lemke, & Okrasa, 1976; Döcke, Rohde, Lange, & Dörner, 1980). In rhesus monkeys, lesions of the entire amygdala at 11 months of age did not influence age at menarche (Norman & Spies, 1981), however the lesions occurred after development of the amygdala, which is complete by 8 months of age (Payne, Machado, Bliwise, & Bachevalier, 2010).

The current study focused on the effects of neonatal amygdala lesions on the timing of menarche and first ovulation in rhesus macaque females reared in large, species-typical social groups. If the amygdala mediates the effects of social context on pubertal timing, then neonatal lesions of the amygdala should result in less variation in age at menarche and first ovulation and the variation in pubertal timing should not be related to social rank. We report here that neurotoxic lesions of the amygdala at one month of age in female rhesus monkeys advanced puberty onset, with menarche occurring when females were approximately 1.5 years of age, but increased the variation in age at first ovulation. Social rank was only related to age at menarche in lesioned females and was not related to age at menarche in control females or first ovulation in lesioned or control females.

Method

Subjects

Female rhesus macaques (*N*=16) living with their mothers and siblings in large, species-typical social groups at the Yerkes National Primate Research Center Field Station (Lawrenceville, GA) were selected for this study. Social groups consisted of approximately 25 adult females and their offspring under three years of age, as well as two adult males. Subjects were infants of mothers in high-, middle-, and low-ranking

matrilines, excluding infants from the alpha and lowest-ranking matrilines. Subjects were housed in 38m x 38m outdoor areas with attached heated and air-conditioned indoor quarters.

Females were assigned to one of three neonatal treatments: neonatal amygdala lesion (Neo-A; n= 7), sham-operated control (Neo-C; n= 6), or behavioral sham control (Neo-BC; n= 3). Neo-A females received MRI-guided bilateral neurotoxic lesions of the amygdala (*M*=27.14±0.74 days of age), whereas Neo-C females (*M*=24.17±1.99 days of age) received a sham surgery, consisting of anesthesia and surgical opening and suturing of the scalp. Neo-BC females (*M*=28.33±1.20 days of age) received 24hr separation from their mothers duplicating the separation of the other two groups of females, and a 2hr period of anesthesia, without any scalp surgery. Once female subjects with their mothers were returned to the group following surgery or behavioral sham manipulations, subjects remained in a social group, except for removal for short experimental procedures or for medical care. One Neo-A female was temporarily housed individually for a two-month period during data collection due to colony management changes in her social group, but neither pubertal event studied occurred during this time period. All procedures were approved by Emory University's Institutional Animal Care and Use Committee and followed the Guide for the Care and Use of Laboratory Animals by the National Institute of Health.

Neonatal Amygdala Lesion (Neo-A) Surgery and Procedure

The amygdala lesion surgery procedure has been previously described (see Raper, Bachevalier, Wallen, & Sanchez, 2013). Briefly, subjects and their mothers were removed from the social group and transported to the Yerkes National Primate Research

Center: Main Center. On the morning of surgery, the infant was separated from the mother, anesthetized (Ketamine hydrochloride, 100mg/ml), intubated and given isoflurane (1-2% to effect) throughout the surgical procedure. Injection site coordinates were determined by securing the female's head in a nonferromagnetic stereotaxic apparatus and using vitamin E filled earbars as reference points in the T1 images. T1-weighted coronal images (spin-echo sequence, echo time [TE] = 11ms, repetition time [TR] = 450ms, contiguous 4mm sections, 12cm field of view [FOV], 256x256 matrix) were taken at 1mm slices throughout the brain. In addition, three fluid attenuated inversion recovery (FLAIR) images (3D T1-weighted fast spoiled gradient [FSPGR]-echo sequence, TE = 2.6ms, TR = 10.2ms, 25 flip angle, contiguous 1mm sections, 12 cm FOV, 256x256 matrix) were completed so that coronal images were taken at 3mm segments throughout the brain.

Four injection sites (1mm dorsal, ventral, medial, and lateral to the center of the amygdala) were determined using the T1 image in which the amygdala was largest. Two or three additional injection sites (1mm lateral and medial or 1mm lateral, medial, and dorsal to the center of the amygdala) were determined using T1 images anterior and posterior to the center of the amygdala. Anterior/posterior and dorsal/ventral coordinates were determined by calculating the distances between the injection site and the starting point of the ear bars, measured by the contrast of the vitamin E on the T1 images. The distances between the intended injection site and the midline of the brain, identified by the third ventricle, were calculated for medial/lateral coordinates. The MRI coordinates were then used to calculate stereotaxic coordinates for the injection sites.

Bilateral craniotomies anterior to bregma and dorsal to the amygdala were performed and the dura was cut to expose the brain for the injections. Bilateral ibotenic acid (PH 7.8-7.9, 10 mg/ml concentration) injections (6-10 injections per hemisphere; 0.6-0.8 µl/injection) occurred simultaneously at each injection site and were manually injected at a rate of 0.2 µl/min. To prevent the ibotenic acid from spreading beyond the amydala, the needle remained in place for three minutes following the completion of each injection, allowing for diffusion of the ibotenic acid before the needle was removed. Upon completion of the injections, the dura was sutured, covered with Surgicel NU-KNIT, and connective tissues were then sutured along the midline.

After recovery from anesthesia, the infant was housed individually in the nursery in an incubator ventilated with oxygen overnight. The next morning, the infant was returned to its mother and continuous monitoring of the pair via a web camera occurred to determine whether the infant was nursing from the mother. Overnight separations with the infant returning to the nursery and morning reunions with the mother occurred until the animal was observed comfortably nursing on its mother to ensure the animal remained healthy after surgery. Infants were supplemented with formula during this period of reunion.

T1 and FLAIR coronal images collected one week after surgery were compared to images prior to surgery to determine the location and extent of the lesion. After the post-surgical MRI scans and when the infant was nursing regularly, the pair was returned to their social group at the Yerkes Field Station, where the pair was closely monitored to ensure both the mother and infant successfully reintegrated into the group.

Sham-Operated (Neo-C) Surgery and Procedure

The same treatments and procedures were performed on sham-operated controls (Neo-C) as were performed on amygdala lesion animals (Neo-A) with the exception of two procedures. Neo-C animals did not have a needle lowered into the amygdala and thus, did not receive any injections into the amygdala and Neo-C animals did not receive post-surgical MRI scans. To control for the time away from the mother Neo-A animals experienced during the post-surgical scans, Neo-C animals were removed from their mother and housed in the nursery for the same amount of time Neo-A animals were separated from their mothers. Following this separation, mother-infant pairs were reunited and transported back to the Yerkes Field Station where the pair was returned to the social group as previously described.

Behavioral Sham (Neo-BC) Procedure

Animals in the Neo-BC group were removed from their social group with their mothers and moved to another building at the Yerkes Field Station were the mother-infant pair was housed for the rest of the procedure. The following day, the infant was separated from its mother, anesthesized, and had its head shaved and cleaned with Nolvasan solution as was the case for infants undergoing surgery. MRI scans or surgical procedures were not performed on Neo-BC animals. Once the infant recovered from anesthesia, the infant received the same treatment as Neo-C animals, which included overnight recovery and housing in an oxygenated incubator, and was returned to its mother the following morning. A second separation from the mother, duplicating the post-lesion separation occurred one week later. After this second separation, mother-infant pairs were reunited and returned to the social group the following day as previously described.

Lesion Assessment

The location and extent of the lesion was determined by comparing the post-surgical FLAIR images to the pre-surgical images, using the hypersignals from the edema shown on the post-surgical images (Raper et al., 2013). Using the program Image-J, the surface area of the damage is calculated for each image and multiplied by 1mm, the thickness of each section, to calculate the volume of damage. The volume of damage is then divided by the total volume of the amygdala, calculated using a template brain, to determine the percentage of damage to the amygdala. Extent of amygdala damage (Table 1) was calculated for the right hemisphere, left hemisphere, average amygdala damage (average of right and left amygdala damage), and amygdala damage shared by both hemispheres (Raper et al., 2013). An example case of the bilateral amygdala lesion extent, using FLAIR images, is presented in Figure 1.

Data Collection Procedure

All subjects were trained as infants to separate from the group and to run into an indoor catch area. Once in the catch area, subjects were transferred to a temporary cage, which contained two small holes, allowing the subjects to extend a leg through an opening. Subjects were habituated to this procedure for a minimum of nine months prior to the start of data collection and this procedure has little effect on endocrine measures (Blank, Gordon, & Wilson, 1983).

Rhesus macaques are seasonal breeders, with ovarian function occurring from late September until March each year, though menarche can occur year round (Wilson et al., 1986; Wilson & Gordon, 1989; Wilson et al., 2013). Vaginal bleeding was assessed and blood was collected at least three times a week from August until March or 45 days after

the last menstruation, whichever occurred later, beginning at 14-17 months of age. Data collection occurred annually until first ovulation was detected. Menstruation was detected by inserting a small, cotton-tipped swab (Puritan 6" cotton tipped applicators) moistened with water into the vagina to check for menstrual blood. Blood collection occurred between 1130h and 1530h to detect luteal increases in progesterone.

Age at Menarche & First Ovulation

Menarche was defined as the first day menstrual blood was detected on the swab and age at menarche was calculated from birth date. Early menarche was defined as reaching menarche during the breeding season at approximately 1.5 years of age, whereas on-time menarche was defined as menarche at approximately 2.5 years of age. First ovulation was defined as progesterone levels above 2ng/ml for a minimum of seven days or above 5ng/ml for at least three days, with date of ovulation calculated as two days prior to this increase in progesterone. Age at first ovulation was calculated based on birth date. One control female had not ovulated by April of the third breeding season, when approximately 4 years of age, and thus, the date when data collection ended was used as the date of first ovulation for this female. Adolescent sterility was calculated by using the difference between age at first ovulation and menarche (Foster, 1977).

Body Weight

Body weights were collected weekly during data collection. Body weight at menarche and first ovulation were calculated by averaging body weights for the four week period prior to the pubertal event. Mean body weight for each calendar month from September-February during the first breeding season was calculated to examine

differences between treatment groups. The overall mean of these monthly body weights was then calculated and used as mean body weight at 1.5 years of age in analyses.

Social Rank

Social rank for juvenile females reflects their mother's social rank and thus, social rank for each animal was calculated at two time points: using the maternal rank at birth and maternal rank in August, at the start of data collection just prior to the breeding season, when subjects were 14-17 months of age (referred to as juvenile social rank). For each animal, social rank was assigned by dividing the rank of the mother by the total number of adult females in the group, which compensates for different group sizes. Several animals (2 Neo-A and 2 Neo-C) were housed in a mixed-sex peer group during the breeding season at 1.5 years of age and juvenile social rank was calculated by dividing the female's social rank by the total number of animals in the group.

Hormonal Assays

Blood samples were collected in EDTA tubes (BD Vacutainer, #366431), centrifuged, and the plasma was stored at -20^{o}C until assayed. Radioimmunoassay commercially prepared kits (Siemens Healthcare, Los Angeles, CA) were used to determine progesterone levels, with a lower sensitivity limit of 0.10ng/ml. Hormone assays were completed by the Biomarkers Core Laboratory at the Yerkes National Primate Research Center.

Statistical Analysis

The proportion of control and lesioned females experiencing menarche and first ovulation each season was compared using a chi-square test. Regression analyses were completed to determine the effect of treatment, body weight, and social rank on age

(days) at menarche and first ovulation. Correlations were performed to examine relationships within treatment groups, between lesion extent and age at menarche/first ovulation, and between body weight at menarche/first ovulation and age at menarche/first ovulation. Monthly body weight during the first breeding season was examined using a repeated measures ANOVA analysis to determine how body weight differed by treatment and year of menarche, using Bonferroni pairwise comparison to identify group differences. Coefficients of variation (*CV*) (standard deviation/mean) were compared between treatment groups using the ratio of squared CVs as an F value to determine if the variation in age at first ovulation differed by treatment (Sokal & Braumann, 1980; Wallen & Lloyd, 2008).

Quantitative data are presented as means and standard errors of the mean (SEM). A $p \leq .05$ is considered significant. Degrees of freedom are reported based on whether homogeneity of variances tests or sphericity tests can be assumed. Effect sizes estimates reported are Cohen's *d* (Cohen, 1969, 1988) for comparisons between 2 groups, η^2 for the repeated measures ANOVA, and φ for Chi-square statistics.

Neither age at menarche ($t(7) = 1.33$, $p = .224$) nor age at first ovulation ($t(7) = 0.73$, $p = .487$) differed between surgical and behavioral shams and thus, these two groups were combined as one control group (Neo-C) for all analyses.

Age at menarche was compared using independent t-tests with menarchal age calculated two different ways because there was a period of intermittent assessment of vaginal bleeding between the first and second breeding season. Because of the intermittent sampling between March and August, we could not say with certainty that menarche did not occur during the period of intermittent sampling in females that did not

experience menarche during the first breeding season. For these females, we analyzed the data first assuming that they had experienced menarche the first day after we stopped consistent sampling and this age was used to compare age of menarche in lesioned and control females. We then compared age at menarche using the age at the first detected menstrual bleeding in the following breeding season, likely, the true menarche. The first method likely markedly underestimates the age of menarche of those females not experiencing menarche in the first breeding season, but is a conservative estimate as it biases against finding a difference in age at menarche. The methods for estimating age of menarche only affected control females as all lesioned females experienced menarche prior to the period of intermittent menstrual sampling.

Results

Menarche

All lesioned females experienced early menarche, during the breeding season at 1.5 years of age, in comparison to only four of nine control females, $X^2(1, N=16) = 5.66$, $p = .017$, $\varphi = .59$ (Figure 2). Five of nine control females experienced menarche on-time during the next breeding season, when approximately 2.5 years of age. Age at menarche was significantly earlier in lesioned females, whether menarcheal age was calculated conservatively (Neo-A: 530.86±20.13 days, Neo-C: 634.33±31.73 days, $t(12.94) = 2.75$, $p = .016$, $d = 1.34$) or using the age at first detection of menstrual bleeding (Neo-A: 530.86±20.13 days, Neo-C:734.44±61.56 days, $t(14) = 2.81$, $p = .014$, $d = 1.71$). Method of calculating age at menarche also did not alter the results of the linear regression when treatment (Neo-A or Neo-C) was used to predict age at menarche (actual age at menarche: $F(1,15) = 7.88$, $p = .014$; conservative age at menarche: $F(1,15) = 6.59$, $p =$

.022). The method of calculating age at menarche did not alter the significance of the findings between lesioned and control females and therefore, the actual age at menarche was used in all analyses. Neo-A females did not differ significantly in age at menarche from the four Neo-C females experiencing early menarche (Neo-A: 530.86±20.13 days; Neo-C: 544.75±27.72; $t(9) = 0.41$, $p = .691$). Treatment (lesion vs. control) significantly predicted age at menarche, $\beta = -.60$, $t(14) = -2.81$, $p = .014$, and accounted for 36% of the variance in age at menarche, $R^2 = .36$, $F(1,15) = 7.88$, $p = .014$. Age at menarche within Neo-A females was significantly related to the amount of damage to the right amygdala (Table 1), with Neo-A females with more right amygdala damage experiencing earlier menarche. Age at menarche was not significantly related to left amygdala damage, average amygdala damage, or shared amygdala damage (Table 1).

Average body weight of all females for the four weeks prior to menarche was positively related to age at menarche, $r(16) = .92$, $p < .001$, in that females reaching menarche earlier weighed less at the time of menarche than did females with a later menarche. Thus, reaching a critical body weight is not required for menarche and does not explain the variation in the age at menarche. In addition to the 36% of variance explained by neonatal treatment, average body weight during the breeding season at 1.5 years of age accounted for an additional 22% of the variance, $\beta = .50$, $t(13) = 2.64$, $p = .02$, with both treatment and body weight at 1.5 years of age accounting for a total of 58% of the variance in age at menarche, $R^2 = .58$, $F(2,15) = 9.12$, $p = .003$. However, this relationship between body weight at 1.5 years of age and age at menarche differed by treatment group, as Neo-C females showed a trend for a positive correlation, $r(9) = .66$, $p = .051$, whereas there was no relationship in Neo-A females, $r(7) = .27$, $p = .565$ (Figure

3). To examine how body weight at 1.5 years differed by year of menarche and treatment, a repeated measures ANOVA was performed for lesioned, early menarchal controls, and on-time menarchal controls. There was a trend for a main effect of monthly body weight (September-February) during the first breeding season, 1.5 years of age, between lesion, early menarchal controls, and on-time menarchal controls, $F(2,13) = 3.753$, $p = .052$, $\eta^2 = .37$ (Figure 4). Follow-up pairwise comparisons using a Bonferroni correction, showed a trend for a difference in body weight between lesion and on-time menarche controls, $p = .081$, $d = 1.75$, but there were no significant differences in body weight between lesion and early menarche controls, $p = 1.0$, $d = 0.05$, or early and on-time menarche controls, $p = .127$, $d = 1.40$. There was not a significant interaction between group (lesion, early menarchal controls, and on-time menarchal controls) and body weight, $F(5.94, 38.57) = 1.05$, $p = .411$, indicating weight gain during the breeding season was consistent for each group and cannot account for the trend in differences in body weight (equal variances were not assumed).

Social rank at birth ($\beta = .057$, $t(13) = 0.25$, $p = .809$) or juvenile social rank ($\beta = .26$, $t(13) = 1.26$, $p = .230$) did not significantly predict age at menarche after using treatment as a predictor variable. Further analysis revealed that social rank at birth, $r(7) = .78$, $p = .04$, and juvenile social rank, $r(7) = .82$, $p = .023$, were both significantly related to age at menarche in Neo-A females in that higher- ranking females reached menarche earlier. However, right lesion extent, which significantly predicted age at menarche, was also related to social rank at birth, $r(7) = -.83$, $p = .02$, and juvenile social rank, $r(7) = -.91$, $p = .004$. Thus, the independent effects of right lesion extent and social rank on age at menarche in lesion females are not clear. Neither the relationship between

age at menarche and social rank at birth, $r(9) = -.04$, $p = .923$, nor age at menarche and juvenile social rank, $r(9) = .27$, $p = .481$, was significant in Neo-C females.

First Ovulation

The proportion of females reaching first ovulation each year did not differ between lesioned and control females, $X^2(2, N=15) = 4.39$, $p = .112$, $\varphi = .54$ (Figure 5). Treatment significantly predicted age at first ovulation and accounted for 30% of the variance in age at first ovulation, $\beta = -.54$, $t(13) = -2.33$, $p = .037$; $R^2 = .30$, $F(1,14) = 5.43$, $p = .037$, with lesion females experiencing earlier first ovulation. Lesion females (*CV*=39.00) had a greater variation in age at first ovulation in comparison to control females (*CV*= 16.69), $F(1,13) = 5.46$, $p = .036$). Age at first ovulation was significantly related to damage to the left amygdala in that females with more left amygdala damage experienced first ovulation later (Table 1). Age at first ovulation was not related to damage to the right amygdala, average amygdala damage, or shared amygdala damage (Table 1).

Body weight at first ovulation was positively related to age at first ovulation, $r(15) = .75$, $p = .001$. Body weight at 1.5 years of age did not significantly account for any additional variance after treatment, $\beta = -.30$, $t(12) = -1.23$, $p = .242$. However, this was due to different directional effects in lesioned and control females (Figure 6). In Neo-C females, average body weight at 1.5 years of age was not a significant predictor of age at first ovulation, though there was a trend for greater body weights predicting earlier age at first ovulation, $\beta = -.59$, $t(7) = -1.92$, $p = .097$; $R^2 = .34$, $F(1,8) = 3.68$, $p = .097$. In contrast to Neo-C females, in Neo-A females (one outlier excluded from analysis), average body weight at 1.5 years of age was a significant predictor of age at first

ovulation, $\beta = .93$, $t(3) = 4.43$, $p = .021$; $R^2 = .87$, $F(1,4) = 19.59$, $p = .021$, in which greater body weight was related to a later age at first ovulation. Excluding the outlier, left amygdala damage and body weight at 1.5 years of age explained 97% of the variance in age at first ovulation in lesioned females, $R^2 = .97$, $F(1,4) = 31.14$, $p = .031$. There was no relationship between left amygdala damage and average body weight when the outlier was included in the analysis, $r(6) = .49$, $p = .323$, or excluded from the analysis, $r(5) = .64$, $p = .241$.

Neither social rank at birth, $\beta = .20$, $t(12) = 0.80$, $p = .439$, nor juvenile social rank, $\beta = .36$, $t(12) = 1.65$, $p = .125$, accounted for any additional variance in age at first ovulation after treatment. Because the effect of social rank may differ between lesioned and control females, the effect of social rank on age at first ovulation was also examined within each treatment group. Age at first ovulation was not related to social rank at birth (Neo-A: $r(6) = .34$, $p = .512$; Neo-C: $r(9) = .16$, $p = .675$) and juvenile social rank (Neo-A: $r(6) = .57$, $p = .235$; $r(9) = .33$, $p = .383$) for either lesioned or control females.

Adolescent Sterility

Treatment did not significantly predict the length of adolescent sterility, $\beta = -.20$, $t(13) = -0.74$, $p = .472$; $R^2 = ..04$, $F(1,14) = 0.55$, $p = .472$ (Figure 7), despite treatment being a significant predictor for age at menarche and first ovulation. Adolescent sterility length was significantly related to age at first ovulation in both Neo-C females, $r(9) = .71$, $p = .033$, and Neo-A females, $r(6) = .99$, $p < .001$, with an earlier age at first ovulation related to a shorter period of adolescent sterility. Age at menarche and length of adolescent sterility were related in Neo-C females, $r(9) = -.68$, $p = .046$, indicating later menarche was related to a shorter duration of adolescent sterility. Although later

menarche and earlier first ovulation were both related to shorter adolescent sterility length in control females, there was no relationship between age at menarche and age at first ovulation, $r(9) = .044$, $p = .911$. The relationship between adolescent sterility length and age at menarche was not found in lesion females, $r(6) = -.05$, $p = .928$.

Discussion

Age at menarche in rhesus monkeys typically occurs at 2.5 years of age in rhesus macaques (Resko et al., 1982; Wilson et al., 1988). Lesions of the amygdala resulted in all females reaching menarche at approximately 1.5 years of age, one year earlier than menarche is typically observed. The proportion of females reaching first ovulation each season did not differ between lesioned and control females, possibly because the variation in age at first ovulation was greater in lesioned females. However, treatment significantly predicted 30% of the variance in age at first ovulation, with lesioned females experiencing an earlier age at first ovulation. These results conflict with data showing bilateral amygdala lesions at 10-13 months of age have no effect on age at menarche or first ovulation (Norman & Spies, 1981). One possible explanation for the lack of an effect found by Norman and Spies is that their lesions were created after amygdala development was complete, at eight months of age (Payne et al., 2010). In the current study, amygdala lesions occurred at approximately one month of age, when the amygdala has the greatest volume increase (Payne et al., 2010). The variation in results based on timing of amygdalectomy is consistent with the data in female rats showing lesions of the anterior MeA at 15 days of age delayed pubertal onset, lesions at 21 days of age resulted in earlier pubertal onset, and lesions at 26 days of age had no effect on pubertal onset (Döcke, 1974; Docke et al., 1976; Döcke et al., 1980). It is also possible that different

environmental conditions may have produced the differing results between the effects of juvenile lesions and our neonatal lesions. Norman & Spies' subjects were housed indoors and all females experienced first ovulation in the same year, when 3.5 years of age, in contrast to the current study where females were exposed to seasonal changes and age at first ovulation was distributed across three seasons. Whether this increased variation in age at first ovulation in the current study is a result of the timing of the lesion, exposure to seasonal elements, or an effect of living in a social group is not clear.

One hypothesis to explain the variation in timing of pubertal onset in humans is that a critical body weight must be reached before menarche occurs (Frisch & Revelle, 1970). We report that body weight at menarche was positively related to age at menarche, indicating a critical body weight is not necessary for menarche to occur, and a similar result was found for age at first ovulation. Greater body weight is related to earlier in menarche in humans and rhesus monkeys (Moffit et al., 1992; Terasawa et al., 2012), but this effect cannot explain earlier menarche in amygdala lesioned females as there was a trend, likely due to small sample sizes, for lesioned females having lower body weights and BMIs during the first breeding season than control females that reached menarche one year later, at 2.5 years of age. The magnitude of the effect of body weight between lesioned and on-time menarchal females was large, $d = 1.75$, and a significant effect would likely be observed with larger sample sizes. Earlier menarche in amygdala lesioned females is not regulated by environmental factors such as greater body weight, which is inconsistent with previous data showing a relationship with greater body weight and earlier menarche (Moffit et al., 1992; Terasawa et al., 2012). There was no significant relationship between body weight at 1.5 years of age and age at first ovulation

in control females, indicating body weight at this age is not predictive of age at first ovulation, which is consistent with data showing body weight prior to 31 months did not differ between females that ovulated at 2.5 or 3.5 years of age (Wilson et al., 1986). Data in rhesus monkeys has either found a positive relationship between body weight and age at first ovulation (Zehr et al., 2005) or no relationship between body weight and age at first ovulation (Wilson et al., 1986), and pubertal data in humans has focused on the relationship between body weight and menarche, not body weight and first ovulation (Moffit et al., 1992; Brooks et al., 1995). Thus, it was unexpected that in Neo-A females, greater body weight at 1.5 years of age was related to later age at first ovulation. Extent of the lesion in the left hemisphere, which was also a significant predictor of age at first ovulation, was not related to body weight at 1.5 years of age, indicating the relationship between body weight and age at first ovulation is not an effect of the amount of left amygdala damage. It is not clear why lower body weight at 1.5 years of age is related to earlier age at first ovulation in lesioned females, considering no relationship between body weight and age at first ovulation was found in control females.

Previous work in socially-housed animals has found no relationship between social rank and age at menarche (Wilson & Kinkead, 2008; Zehr et al., 2005), consistent with our control females. By contrast, we found an unexpected relationship between social rank and age at menarche in lesion females, but this relationship is confounded by the finding that extent of lesion damage to the right amygdala is related to both social rank and age at menarche. It is not likely that lesion surgery influenced this relationship between juvenile social rank and age at menarche because both social rank at birth and juvenile social rank were related to both age at menarche and right lesion damage,

indicating that lesion surgery did not alter social rank. Therefore, it is not possible to identify the independent effects that right amygdala damage and social rank have on age at menarche. Low social rank has been previously found to delay age at first ovulation, with all low-ranking females experiencing first ovulation at 3.5 years of age, whereas high- and middle-ranking females reached first ovulation at 2.5 or 3.5 years of age (Zehr et al., 2005). It is possible that the lack of a relationship between social rank and age at first ovulation in this study is due to fewer females in the bottom third of the social hierarchy. We predicted that social rank would be related to age at first ovulation in control females, but not in lesioned females, indicating that the amygdala mediates the relationship between social context and first ovulation. However, the lack of an effect of social rank on first ovulation in control females makes it difficult to examine whether the amygdala modulates social information and alters pubertal timing.

Lesion status significantly predicted age at menarche and age at first ovulation, with lesion females experiencing earlier timing of pubertal events. The length of adolescent sterility however, did not differ between lesion and control females, which suggests that earlier pubertal onset did not alter the relative timing of pubertal events after menarche, but rather simply advanced the timing of the pubertal period in lesioned females. However, age at menarche was not related to age at first ovulation in lesioned or control females, indicating that there is still some variability in the length of the pubertal period and this variability was present in both lesioned and control females.

Neonatal lesions of the amygdala result in earlier menarche and first ovulation, but the exact mechanism(s) resulting in earlier menarche and first ovulation are unknown. One potential mechanism involves the inhibitory effects of *gamma*-Aminobutyric acid,

GABA, release. Prior to puberty, there is a decline in GABA release at approximately the time when GnRH release is increasing (Mitsushima, Hei, & Terasawa, 1994). Administration of bicuculine, a GABA receptor antagonist, to the median eminence in fifteen month old rhesus macaques results in an increase in GnRH release and chronic treatment (15 months-2nd ovulation) using this antagonist results in earlier menarche and first ovulation, with menarche and first ovulation occurring about one year earlier than controls, at 18 months and 30 months respectively (Keen, Burich, Mitsuhima, Kasuya, & Terasawa, 1999). The amygdala contains GABAergic neurons (Pitkänen & Amaral, 1994) and therefore, neonatal lesions of the amygdala may result in decreased GABA release, thereby resulting in an earlier increase in GnRH, earlier menarche, and ultimately, earlier first ovulation. Future research is needed evaluate this hypothesis and to fully understand the mechanism by which the amygdala is able to influence pubertal timing. In conclusion, the current study demonstrates the amygdala can influence the HPG axis, possibly by altering GnRH release, and damage to the amygdala early in life can result in earlier pubertal onset and reproductive maturity.

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References

Blank, M. S., Gordon, T. P., & Wilson, M. E. (1983). Effects of capture and venipuncture on serum levels of prolactin, growth-hormone and cortisol in outdoor compound-housed female rhesus monkeys (*Macaca mulatta*). *Acta Endocriniologica*, *102*, 190-195.

Cohen, J. (1969). *Statistical Power Analysis for the Behavioral Sciences, 1st Edition*. Hillsdale: Lawrence Erlbaum Associates, (2nd Edition, 1988).

Döcke, F. (1974). Differential effects of amygdaloid and hippocampal lesions on female puberty. *Neuroendocrinology*, *14*, 345-350.

Döcke, F., Lemke, M., & Okrasa, R. (1976). Studies on the puberty-controlling function of the mediocortical amygdala in the immature female rat. *Neuroendocrinology*, *20*, 166-175.

Döcke, F., Rohde, W., Lange, T., & Dörner, G. (1980). Evidence for direct central nervous inhibition of LH secretion during sexual maturation of female rats. *Endokrinologie*, *75*, 1-7.

Ersoy, B., Balkan, C., Gunay, T., & Egemen, A. (2005). The factors affecting the relation between menarcheal age of mother and daughter. *Child: Care, Health, &*

Development, *31*(3), 303-308.

Foster, D. L. (1977). Luteinizing hormone and progesterone secretion during sexual maturation of the rhesus monkey: short luteal phases during initial menstrual cycles. *Biology of Reproduction*, *17*, 584-590.

Frisch, R. E., & Revelle, R. (1970). Height and weight at menarche and a hypothesis of critical body weights and adolescent events. *Science*, *169*, 397-399.

Graber, J. A., Brooks-Gunn, J., & Warren, M. P. (1995). The antecedents of menarcheal age: Heredity, family environment, and stressful life events. *Child Development*, *66*, 346-359.

Grumbach, M. M., & Styne, D. M. (2003). Puberty: Ontogeny, neuroendocrinology, physiology and disorders. In P. R. Larsen, H. M. Kronenberg, S. M. Melmed, & K. S. Polonsky (Eds.), *Williams textbook of endocrinology* (10^{th} ed., pp. 1115-1286). Philadelphia: Saunders.

Herman-Giddens, M. E. (2006). Recent data on pubertal milestones in United States children: the secular trend toward earlier development. *International Journal of Andrology*, *29*, 241-246.

Keen, K. L., Burich, A. J., Mitsushima, D., Kasuya, E., & Terasawa, E. (1999). Effects of

pulsatile infusion of the $GABA_A$ receptor blocker bicuculline on the onset of puberty in female rhesus monkeys. *Endocrinology*, *140*, 5257-5266.

Kling, A., & Cornell, R. (1971). Amygdalectomy and social behavior in the caged stump-tailed macaque (*M. speciosa*). *Folia Primatologica*, *14*, 91-103.

Marshall, W. A., & Tanner, J. M. (1969). Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood*, *44*, 291-303.

Mitsushima, D., Hei, D. L., & Terasawa, E. (1994). γ-Aminobutyric acid is an inhibitory neurotransmitter restricting the release of luteinizing hormone-releasing hormone before the onset of puberty. *Proc Natl Acad Sci U S A*, *91*, 395-399.

Moffit, T. E., Caspi, A., Belsky, J., & Silva, P. A. (1992). Childhood experience and the onset of menarche: A test of a sociobiological model. *Child Development*, *63*, 47-58.

Morris, D. H., Jones, M. E., Schoemaker, M. J., Ashworth, A., & Swerdlow, A. J. (2011). Secular trends in age at menarche in women in the UK born 1908-93: results from the Breakthrough Generation Study. *Paediatric and Perinatal Epidemiology*, *25*(4), 394-400.

Norman, R. L., & Spies, H. G. (1981). Brain lesions in infant female rhesus monkeys:

Effects on menarche and first ovulation and on diurnal rhythms of prolactin and cortisol. *Endocrinology*, *108*(5), 1723-1729.

Payne, C., Machado, C. J., Bliwise, N. G., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in *Macaca mulatta*: A volumetric magnetic resonance imaging study. *Hippocampus*, *20*, 922-935.

Petrulis, A., & Johnston, R. E. (1999). Lesions centered on the medial amygdala impair scent-marking and sex-odor recognition but spare discrimination of individual odors in female golden hamsters. *Behavioral Neuroscience*, *113*(2), 345-357.

Pitkänen, A., & Amaral, D. G. (1994). The distribution of GABAergic cells, fibers, and terminals in the monkey amygdaloid complex: an immunohistochemical and in situ hybridization study. *The Journal of Neuroscience*, *14*(4), 2200-2224.

Raper, J., Bachevalier, J., Wallen, K., and Sanchez, M. (2013). Neonatal amygdala lesions alter basal cortisol levels in infant rhesus monkeys. *Psychoneuroendocrinology*, *38*(6), 818-829.

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*, 354-361.

Rosvold, H. E., Mirsky, A. F., & Pribram, K. H. (1954). Influence of amygdalectomy on social behavior in monkeys. *Journal of Comparative Physiology & Psychology*, *47*, 173-178.

Sokal, R. R., & Braumann, C. A. (1980). Significance tests for coefficients of variation and variability profiles. *Systematic Zoology*, *29*, 50-66.

Terasawa, E., Kurian, J. R., Keen, K. L., Shiel, N. A., Colman, R. J., & Capuano, S. V. (2012). Body weight impact on puberty: Effects of high-calorie diet on puberty onset in female rhesus monkeys. *Endocrinology*, *153*, 1696-1705.

Thompson, C., Schwartzbaum, J. S., & Harlow, H. F. (1969). Development of social fear after amygdalectomy in infant rhesus monkeys. *Physiology & Behavior*, *4*, 249-254.

Wallen, K., & Lloyd, E. A. (2008). Clitoral variability compared with penile variability supports nonadaptation of female orgasm. *Evolution & Development*, *10*, 1-2.

Wierson, M., Long, P. J., & Forehand, R. L. (1993). Toward a new understanding of early menarche: The role of environmental stress in pubertal timing. *Adolescence*, *28*, 913-924.

Wilson, M. E., Bounar, S., Godfrey, J., Michopoulos, V., Higgins, M., & Sanchez, M.

(2013). Social and emotional predictors of the tempo of puberty in female rhesus monkeys. *Psychoneuroendocrinology, 38*, 67-83.

Wilson, M. E., & Gordon, T. P. (1989). Season determines timing of first ovulation in rhesus monkeys (*Macaca mulatta*) housed outdoors. *J Reprod Fertil*, *86*, 435-444.

Wilson, M. E., Gordon, T. P., & Collins, D. C. (1986). Ontogeny of luteinizing hormone secretion and first ovulation in seasonal breeding rhesus monkeys. *Endocrinology, 118*, 293-301.

Wilson, M. E., Gordon, T. P., Rudman, C. G., & Tanner, J. M. (1988). Effects of a natural versus artificial environment on the tempo of maturation in female rhesus monkeys. *Endocrinology, 123*, 2653-2661.

Wilson, M. E., & Kinkead, B. (2008). Gene-environment interactions, not neonatal growth hormone deficiency, time puberty in female rhesus monkeys. *Biol Reprod, 78*, 736-743.

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. *Biol Reprod, 72*, 1087-1094.

	Amygdala Damage			
Subjects	% Right	% Left	Average Right and Left	Shared Right and Left
Neo-A-F1	82.3	0.0	41.2	0.0
Neo-A-F2	65.7	98.7	82.2	64.8
Neo-A-F3	100.0	32.2	66.1	32.2
Neo-A-F4	90.8	89.3	90.1	81.1
Neo-A-F5	61.6	58.4	60.0	36.0
Neo-A-F6	100.0	97.6	98.8	97.6
Neo-A-F7	98.3	99.0	98.6	97.3
Mean Damage	85.5	67.9	76.7	58.4
Age at Menarche	r (7) = -.82, p = .023*	r (7) = -.34, p = .455	r (7) = -.62, p = .141	r (7) = -.53, p = .222
Age at First Ovulation	r (6) = -.50, p = .311	r (6) = .85, p = .031*	r (6) = .62, p = .191	r (6) = .66, p = .153

Table 1. Extent of amygdala lesion damage for the right hemisphere (%Right), left hemisphere (%Left), average damage to the right and left hemisphere (Average Right and Left), and damage shared by both hemispheres (Shared Right and Left) for each subject and the relationship between right, left, average, and shared damage and the timing of menarche and first ovulation. Lesion extent data modified from Raper et al., 2013. First ovulation data on Neo-A-F6 were not available. * indicates significant relationship, $p<.05$.

Figure Captions

Figure 1. FLAIR images for subject Neo-A F4 demonstrating hypersignals as a result of cell death from the ibotenic acid in comparison to T1 images for Neo-C F4.

Figure 2. Age (days) at menarche for lesion and control females. 1.5 years of age=548 days; 2.5 years of age=913 days.

Figure 3. Relationship between average body weight during the breeding season (September-February) when approximately 1.5 years of age and age at menarche for control and lesioned females.

Figure 4. Average monthly body weight during the breeding season when approximately 1.5 years of age for lesioned, early menarchal, and on-time menarchal females. # indicates $p < 0.10$.

Figure 5. Age (days) at first ovulation for Neo-A and Neo-C females. 1.5 years=548 days; 2.5 years=913 days; 3.5 years=1278 days.

Figure 6. Mean body weight at 1.5 years of age and age at first ovulation for Neo-A and Neo-C females.

Figure 7. Length of adolescent sterility, the time between menarche and first ovulation, for lesioned and control females.

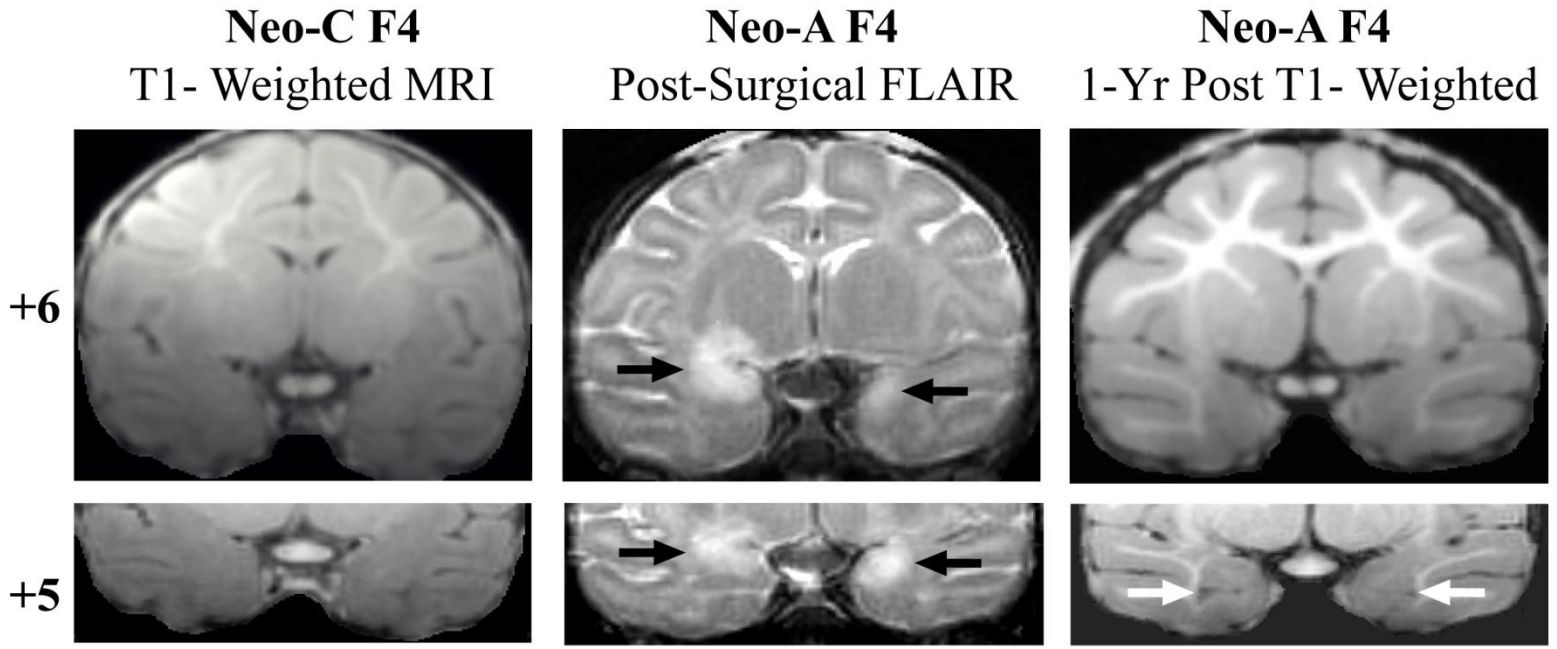
Neo-C F4
T1- Weighted MRI
Neo-A F4
Post-Surgical FLAIR
Neo-A F4
1-Yr Post T1- Weighted
+6
+5

Figure 1.

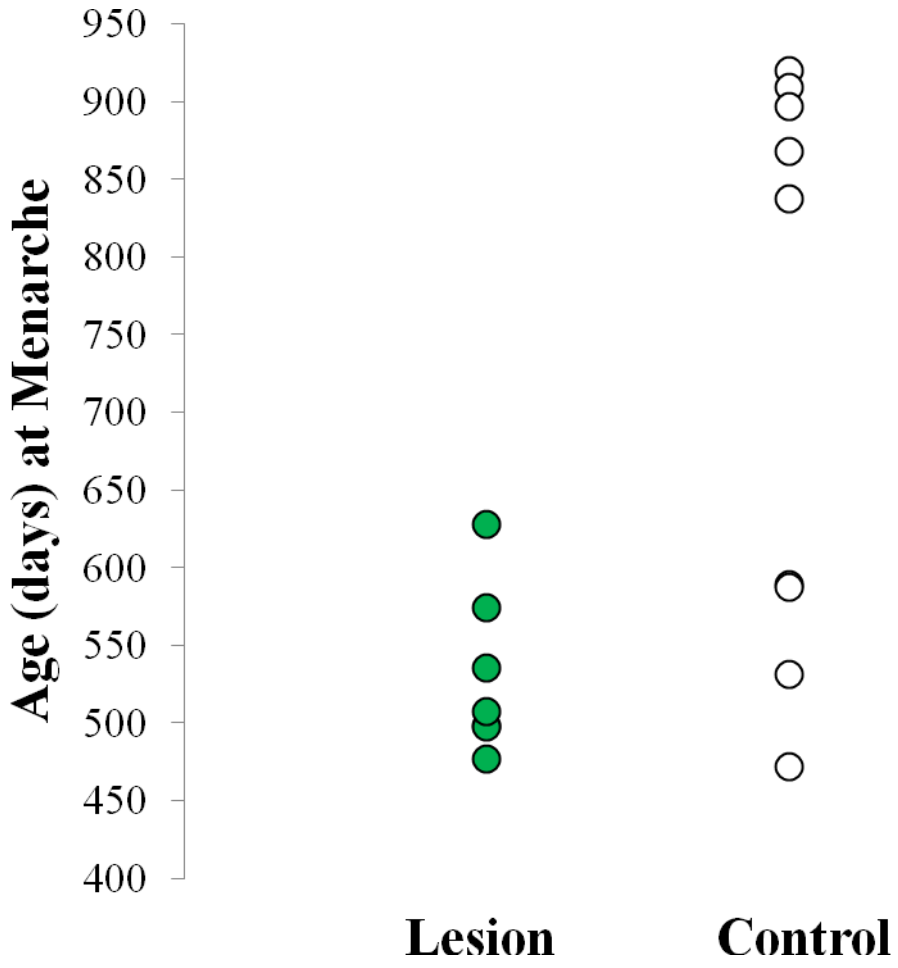
950
900
850
800
750
700
650
600
550
500
450
400
Age (days) at Menarche
Lesion
Control

Figure 2.

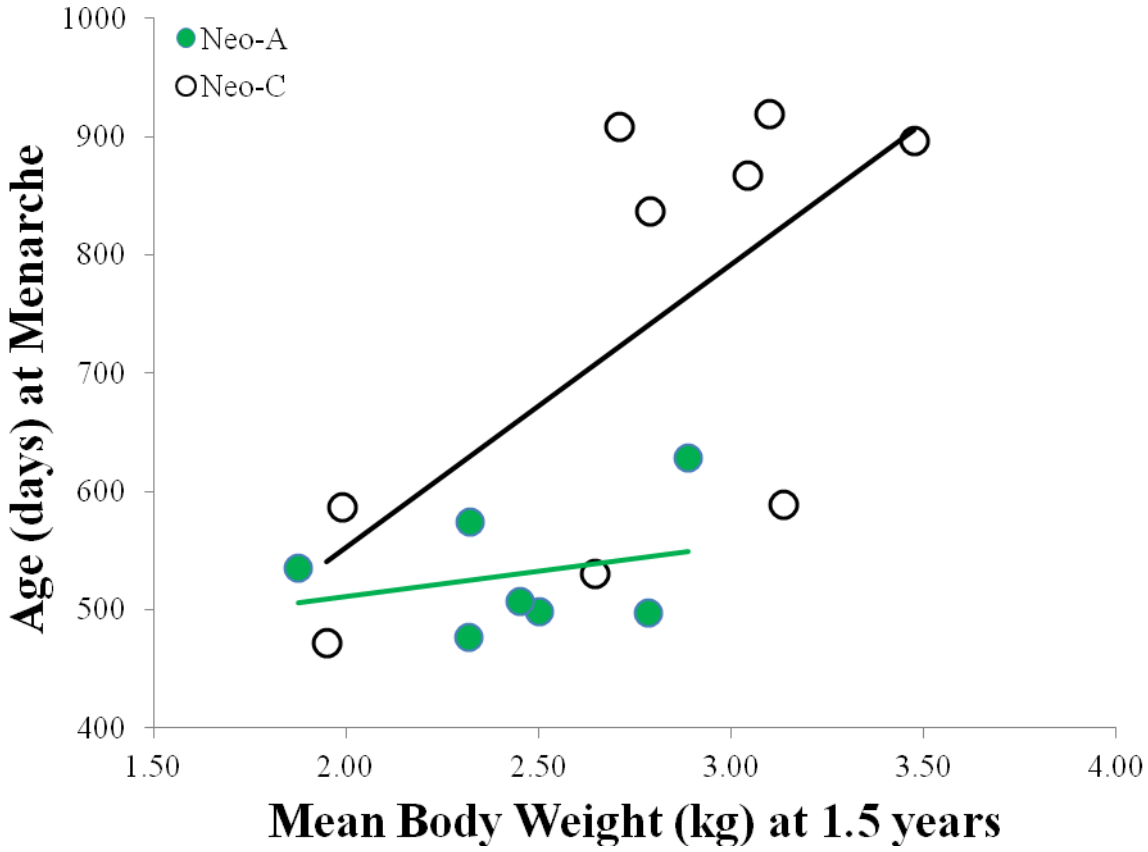
1000
900
800
700
600
500
400
Neo-A
Neo-C
Age (days) at Menarche
1.50
2.00
2.50
3.00
3.50
4.00
Mean Body Weight (kg) at 1.5 years

Figure 3.

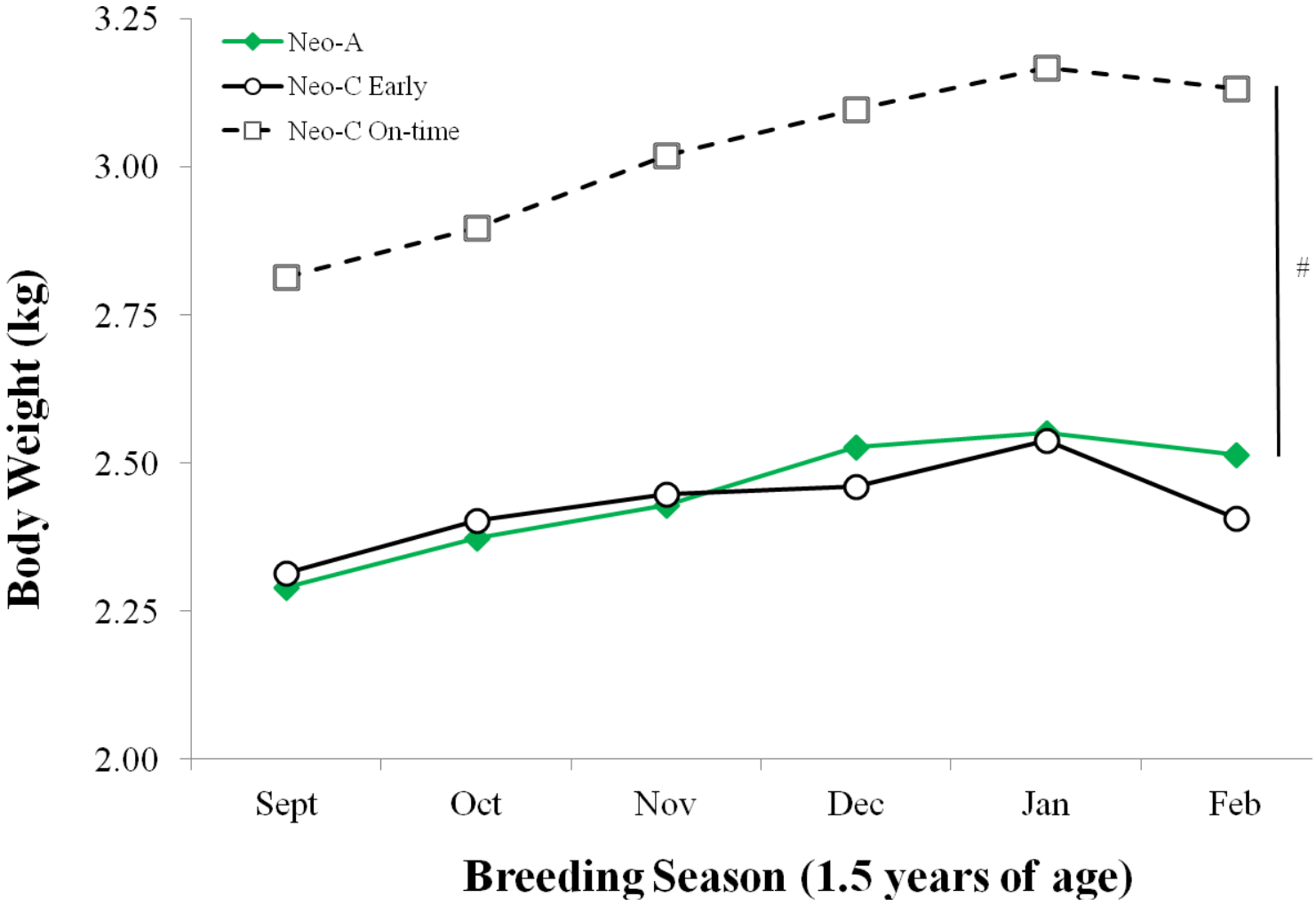
3.25
3.00
2.75
2.50
2.25
2.00
Body Weight (kg)
Neo-A
Neo-C Early
Neo-C On-time
#
Sept
Oct
Nov
Dec
Jan
Feb
Breeding Season (1.5 years of age)

Figure 4.

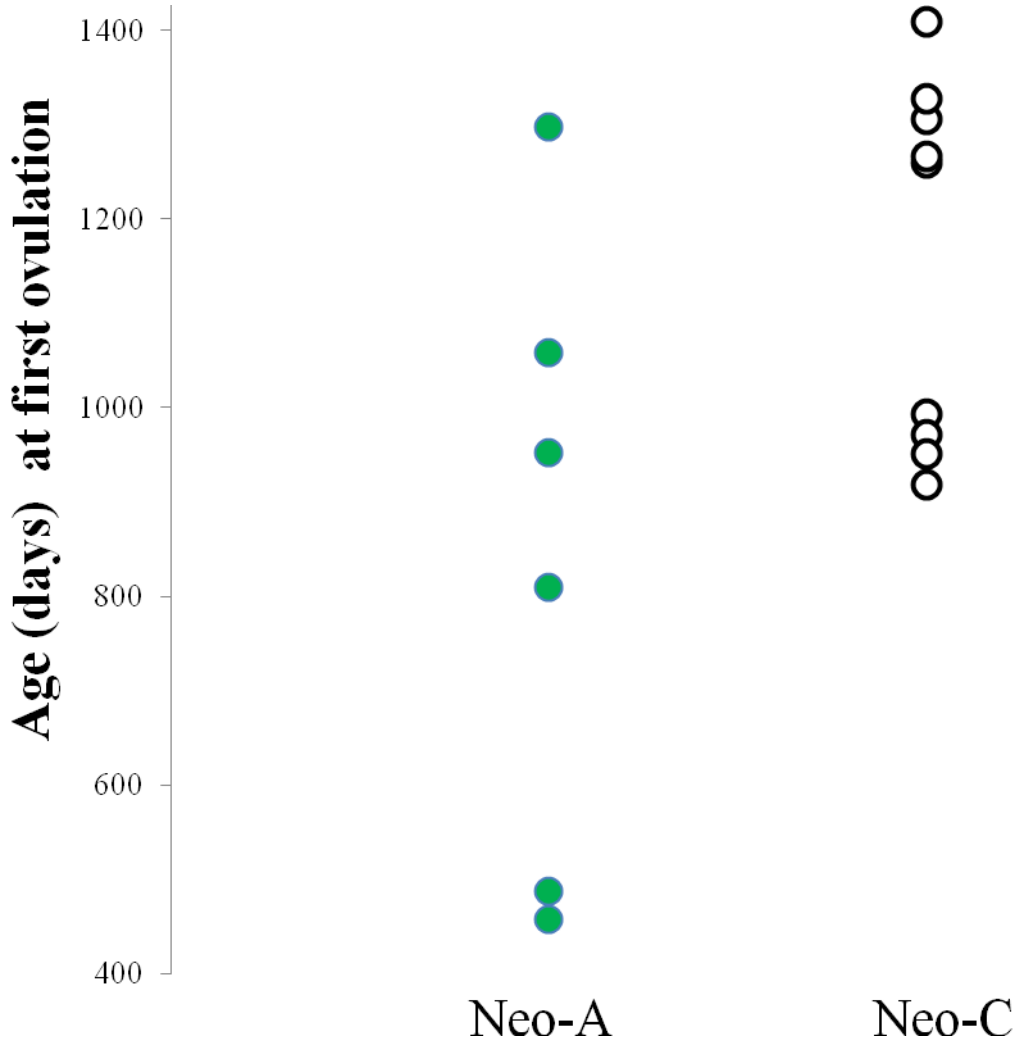
Age (days) at first ovulation
1400
1200
1000
800
600
400
Neo-A
Neo-C

Figure 5.

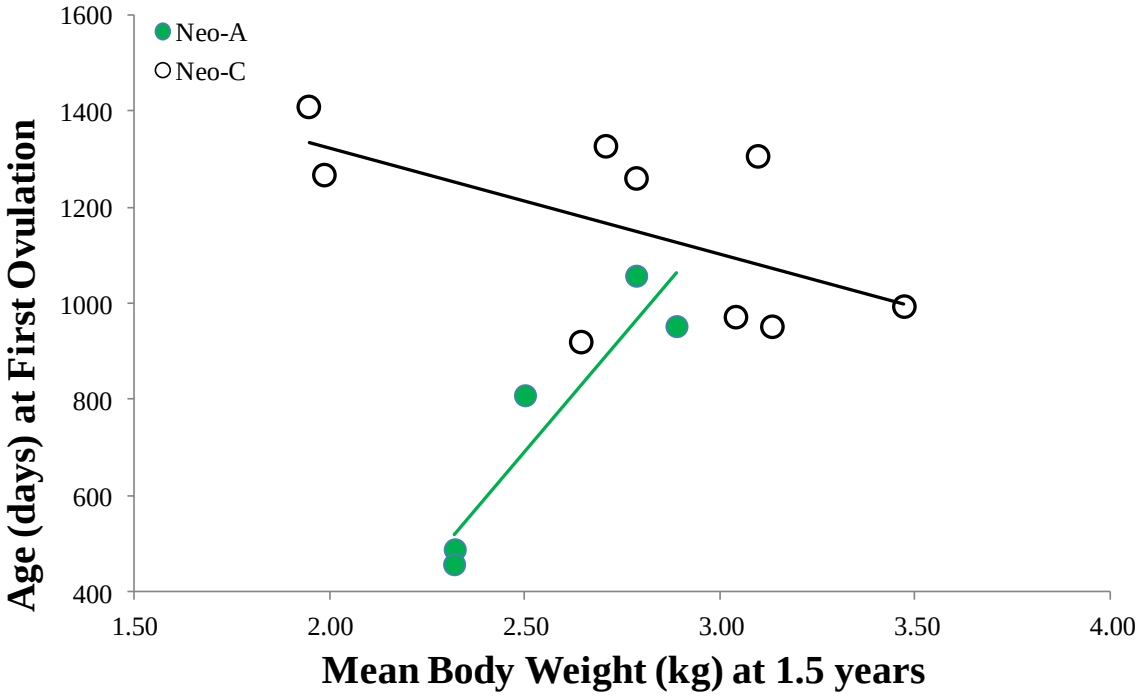
Neo-A
Neo-C
Age (days) at First Ovulation
1600
1400
1200
1000
800
600
400
1.50
2.00
2.50
3.00
3.50
4.00
Mean Body Weight (kg) at 1.5 years

Figure 6.

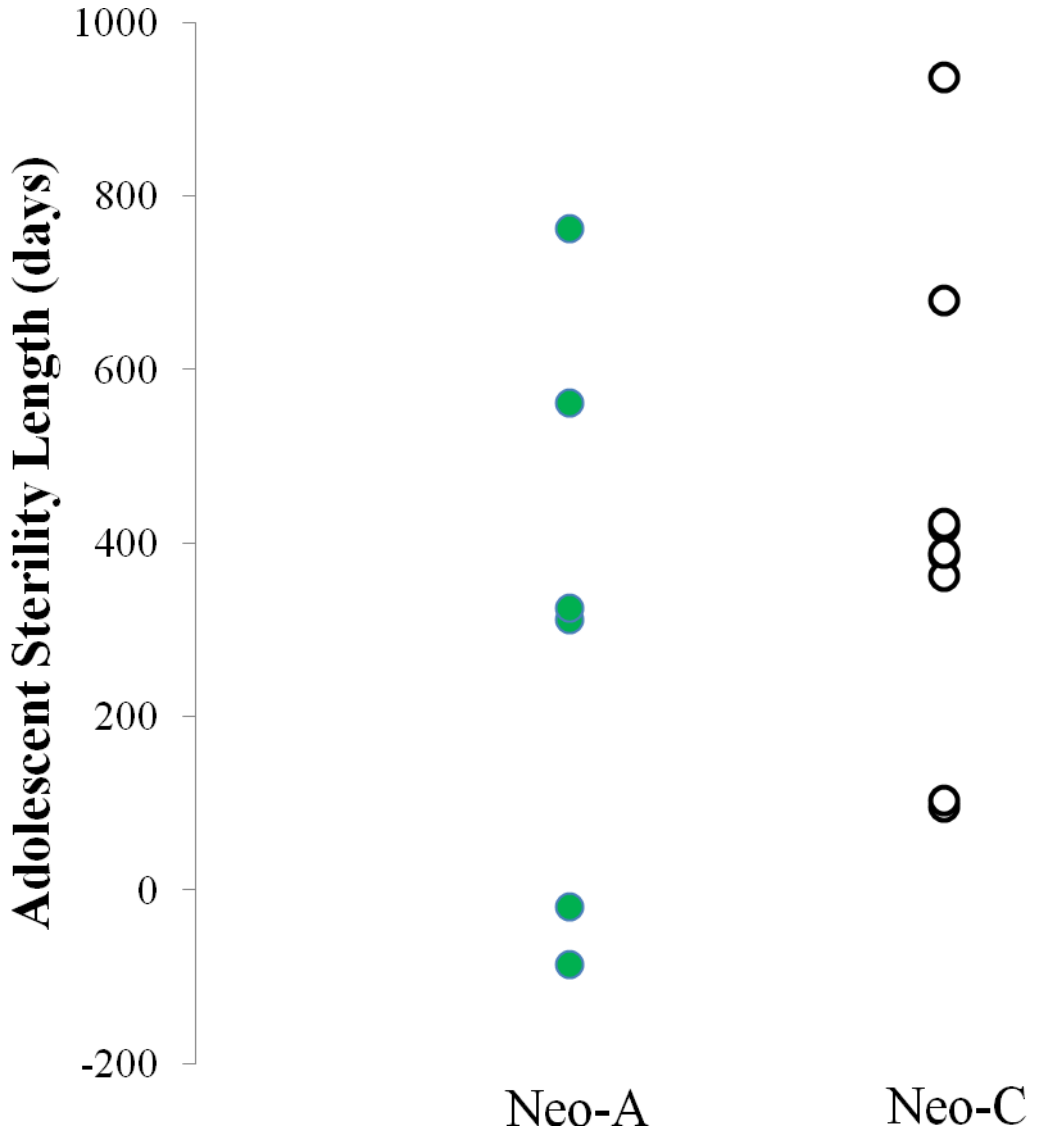
1000
800
600
400
200
0
-200
Adolescent Sterility Length (days)
Neo-A
Neo-C

Figure 7.

Running Head: Neonatal Amygdala Lesions and Sexual Behavior

Effects of Neonatal Amygdala Lesions and Social Context on Sexual Behavior in Female Rhesus Macaques

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Abstract

Sexual behavior is regulated by both physiological and environmental factors, including social context. In female rhesus macaques, social group structure influences the expression of sexual behavior. In pair tests, one male and one female, mounting is observed consistently throughout the menstrual cycle (Goy, 1979). In multi-female groups with one male, sexual behavior is limited to the time of the estradiol peak (Wallen et al., 1984), whereas in multi-female/multi-male groups, sexual behavior is high throughout the first half of the menstrual cycle (Wilson et al., 1982). The brain regions modulating the effects of social context on sexual behavior are unknown. The amygdala is important for social recognition (Bennett et al., 2002; Spiteri et al., 2010), species-typical behavioral responses to social cues (Thompson et al., 1969; Kling & Cornell, 1971), and has afferent and efferent projections to the hypothalamus (Amaral et al.a, 1992) and thus, may modulate the effects of social context on sexual behavior. In the current study, female rhesus macaques received either bilateral neurotoxic lesions of the amygdala or sham-operated procedures at one month of age. Sexual behavior was examined in these females in both a pair test, at 2.5 years of age, and in the social group, at 3.5 years of age. Lesioned females showed higher levels of female-initiated male-directed behaviors than control females, whereas control females showed higher levels of female-initiated behaviors in the social group observations. Despite any differences in female-initiated behavior, female sexual receptivity did not differ between lesioned and control females. Thus, lesions of the amygdala influence female-initiated sexual behavior, but the direction of these effects is dependent on the social context. Estradiol levels were related to some female-initiated behaviors in control females, but not in

lesioned females, which may be a result of reduced sensitivity to estradiol, thereby altering sexual motivation in lesioned females.

Female reproduction is regulated by circulating gonadal hormone levels during the ovulatory cycle. In order for ovulation to occur, estradiol levels must be high enough to induce a surge in luteinizing hormone (LH), which results in ovulation (Butcher, Collins, & Fugo, 1974; as reviewed by Knobil, 1974). Circulating gonadal hormones are also important for the expression of female sexual behavior. In rats estradiol is required for the lordosis response (Pfaff, 1970), which allows the male to copulate with the female. In nonhuman primates and humans, estradiol is not required to make it possible to engage in sexual behavior, but sexual behavior occurs more often when estradiol levels are elevated (Wilson, et al., 1982; Wallen, et al., 1984; Wallen, 1990; Van Goozen et al., 1997). The modulation of sexual behavior by gonadal hormones couples the expression of sexual behavior with maximal fertility allowingsperm to fertilize the mature ovum.

Though circulating estradiol levels modulates sexual behavior, external environmental factors, such as social context, also influence the expression of sexual behavior. In female rats, lordosis responses decrease after females receive repeated intromissions, unless the female can control the timing of intromissions by leaving the male following mounts or intromissions (Bermant & Westbrook, 1966). Thus, female sexual receptivity is altered depending on the female's ability to regulate the pace of mating interactions. In female rhesus macaques social context modulates the relationship between hormones and behavior (Wallen, 1990). Sexual behavior was observed on every day of the menstrual cycle and was weakly coupled to ovarian function when a single female was tested with a single male (pair-tests; Goy, 1979). By contrast, when multiple females lived in a social group with one male, female sexual behavior was strongly coupled to estradiol levels (Wallen, Winston, Gaventa, Davis-DaSilva, & Collins, 1984).

In multi-male/multi-female social groups, high levels of female sexual behavior were observed in the follicular and peri-ovulatory phases of the menstrual cycle, but female sexual behavior decreased during the luteal phase, as was the case with the single male, multiple female groups (Wilson, Gordon, & Collins, 1982).

In addition to social group structure, female social rank influences sexual behavior in that high-ranking females initiate sexual behavior more often than low-ranking females during the follicular phase. High-ranking females engage in high amounts of sexual behavior throughout the follicular and periovulatory phases and consequently, show no significant correlation between their estradiol levels and initiation of proximity to males during the follicular phase of the menstrual cycle (Wallen, 1990). In contrast to high-ranking females, low-ranking females approach and solicit adult males at high rates only around the time of their estradiol peak, behavior that is strongly correlated with changes in estradiol (Wallen, 1990). For a low-ranking female, competing with a high-ranking female to get close to a male is risky and only occurs when female sexual motivation is at its highest, that is when estradiol levels are high (Wallen, 1999). Thus, the relationship between hormonal changes and female sexual behavior is strongly affected by social context; however, the brain region(s) that integrate social contextual information and consequently alter female sexual behavior is unknown.

The amygdala is important for social recognition, integrating social information, and species typical behavioral responses (Thompson, Schwartzbaum, & Harlow, 1969; Kling & Cornell, 1971; Bennett, Greco, Blasberg, & Blaustein, 2002; Spiteri, Musatov, Ogawa, Ribeiro, Pfaff, & Agmo, 2010). In addition, the amygdala has afferent and efferent projections to regions of the hypothalamus important for the expression of sexual

behavior, such as the ventromedial hypothalamus (Mathews & Edwards, 1977; Mathews, Donovan, Hollingsworth, Hutson, & Overstreet, 1983; Yahr & Greene, 1992; Oomura, Aou, Koyama, Fujita, & Yoshimatsu, 1988; as reviewed by Amaral, Price, Pitkänen, & Carmichael, 1992). Thus, the amygdala is one candidate brain region for modulating sexual behavior based on the social context.

In adult female rats, lesions of the medial amygdala, specifically the anterior medial amygdala, decreased the lordosis response when females were not able to pace the timing of mating interactions, but when females were able to pace the timing of mating events, medial amygdala lesions had no effect on lordosis response (Mascó & Carrer, 1980; Guarraci, Megroz, & Clark, 2004; Kondo & Sakuma, 2005). Thus, in rodents, the medial amygdala facilitates female sexual receptivity in social contexts that may not result in the maximum frequency of sexual behavior.

Lesions of the entire amygdala in adult female rhesus macaques impaired aspects of female-initiated behaviors, such as initiating proximity to the male, but had no effect on male-initiated behaviors or female sexual receptivity when females were tested in pair-tests (Spies, Norman, Clifton, Ochsner, Jensen, & Phoenix, 1976). Female sexual receptivity was not altered following bilateral lesions of the amygdala (Spies et al., 1976), but this may reflect the use of pair-tests in which mating is more regulated by male sexual initiation and less influenced by female behavior (Wallen, 1990). It cannot be ruled out that the lack of changes in sexual receptivity following amygdalectomy reflects a lack of amygdala involvement in sexual receptivity.

In groups with multiple females and one or more males, female-initiation of sexual behavior and copulation only occurs during the follicular phase of the menstrual

cycle (Wilson et al., 1982; Wallen et al., 1984). The finding that amygdala-lesioned adult females showed a decrease in female-initiated proximity (Spies et al., 1976) under social conditions where detecting female initiation is difficult (Wallen, 1999)) suggests it is likely that amygdala-lesioned females would show a greater decrease in female-initiated sexual behaviors when observed in large, multi-male/multi-female social groups. It is also expected that lesioned females will receive fewer male-initiated sexual behaviors, such as mounts, because in such complex social groups females initiate almost all sexual interactions (Wallen et al., 1984). Thus, decreases in sexual behavior in lesioned females may best be observed in females living in social groups, with little sexual activity expected to occur in lesioned females. The current study examined the effects of neonatal bilateral neurotoxic lesions of the amygdala on female sexual behavior, when behavior was observed in a social group as well as in pair tests.

Method

Subjects

Sexual behavior was examined in female rhesus macaques (*N*=14) reared in large, species-typical social groups at the Yerkes National Primate Research Center Field Station (Lawrenceville, GA) at approximately 2.5 and 3.5 years of age. Females were from high-, middle-, and low-ranking matrilines and at the time of group behavioral observations, social groups contained 9-23 adult females, 2-3 adult males, and up to 40 juveniles under the age of 3. Subjects were housed in outdoor enclosures with access to an indoor area.

As infants, females were assigned to one of the following neonatal treatment groups: neonatal amygdala lesion (Neo-A; *n*= 5), sham-operated control (Neo-C; *n*= 6),

or behavioral sham control (Neo-BC; *n*= 3). At approximately one month of age, Neo-A females received MRI-guided bilateral neuortoxic lesions of the amygdala, Neo-C females received a sham-operated surgery, and Neo-BC females, though no surgery was performed, received similar experimental procedures.

Neonatal Amygdala Lesion (Neo-A) Surgery and Procedure

Surgical details have been described previously (see Raper, Bachevalier, Wallen, & Sanchez, 2013) and are briefly described here. Prior to surgery, Neo-A infants and their mothers were transported to the main Yerkes National Primate Research Center facility (Atlanta, GA). Approximately three days later, the infant was separated from its mother, anesthesized (Ketamine hydrochloride, 100mg/ml), intubated, and remained sedated with isoflurane (1-2% to effect) throughout the surgical procedure. Using a stereotaxic apparatus to secure the subject's head, T1-weighted coronal images (spin-echo sequence, echo time [TE] = 11ms, repetition time [TR] = 450ms, contiguous 4mm sections, 12cm field of view [FOV], 256x256 matrix) and three fluid attenuated inversion recovery (FLAIR) images (3D T1-weighted fast spoiled gradient [FSPGR]-echo sequence, TE = 2.6ms, TR = 10.2ms, 25 flip angle, contiguous 1mm sections, 12 cm FOV, 256x256 matrix) were performed to determine injection coordinates.

Using the T1 image, four injections sites (1mm dorsal, ventral, medial, and lateral) and an additional two (1 mm lateral and medial) or three injection sites (1mm lateral, medial, and dorsal) were calculated from the center of the amygdala. Injection site coordinates were determined by measuring the distance from the intended injection site to the following reference points: the starting point of vitamin E filled ear bars, measured by the contrast of the vitamin E on the T1 images, for both the

anterior/posterior and dorsal/ventral coordinates, and the midline of the brain, identified by the third ventricle, for medial/lateral coordinates. Bilateral injections (0.6-0.8 μl/injection: 0.2 μl/min) of ibotenic acid (PH 7.8-7.9, 10 mg/ml concentration) were manually given and the needle was removed three minutes after the injection was complete to ensure diffusion of the acid prior to removing the needle.

Following surgery, the infant was housed in an incubator overnight before returning to its mother the next morning. Using a web camera, the pair was monitored during the day and overnight separations continued until the infant was observed nursing. Post-surgical T1 and FLAIR images were completed one week after surgery and these images were compared to pre-surgical images to verify the lesion location and extent. Once the post-surgical MRI scans were obtained, the mother and infant were returned to the Yerkes Field Station and reintroduced to their social group.

Sham-Operated (Neo-C) Surgery and Procedure

Neo-C subjects experienced a similar procedure as Neo-A subjects, including surgery, except that Neo-C subjects did not have a needle lowered into the amygdala. Post-surgical MRI scans were not completed on Neo-C subjects, but Neo-C subjects were temporarily separated from their mother one week after surgery, similar to Neo-A subjects, to minimize treatment group differences in time separated from the mother. With the exception of these two differences, the procedure for Neo-A and Neo-C subjects was the same.

Behavioral Sham (Neo-BC) Procedure

The procedures for Neo-BC subjects were similar to that of Neo-C subjects, but differed in two ways. Neo-BC subjects were not transported to Yerkes Main Center, but

rather were transferred to an indoor-housing area, similar to housing at the Main Center, at the Field Station. Secondly, Neo-BC subjects did not have surgery, but their heads were shaved and cleaned with Nolvasan solution. Other than these two differences, procedures for Neo-C and Neo-BC subjects were the same.

Lesion Assessment

Lesion extent was calculated by using the hypersignals from the edema on the one-week post-surgical FLAIR images and comparing these images to pre-surgical images (Raper et al., 2013). Figure 1 demonstrates an example of the hypersignals on the FLAIR images of Neo-A females. Volume of lesion damage is determined by calculating the surface area of damage on each FLAIR image, multiplied by 1mm to account for the thickness of each image. Percent damage to the amygdala was determined by dividing the volume of damage to the amygdala by total amygdala volume, which was determined using a template brain.

Behavioral Observation Procedure

Rhesus macaques are seasonal breeders, with ovarian cycles limited to the breeding season (September-February; Wilson et al., 1986; Wilson & Gordon, 1989). Behavioral observations were limited to the breeding season and focused on female-initiated behaviors such as solicits and presents, mounting, as well as aspects of proximity, and other submissive and aggressive behaviors (see Table 1 for a complete list and description of behaviors). Observations were completed using the program WinObs, which records real-time data sequentially in actor, behavior, recipient, elapsed time format allowing the determination of the frequency and duration of behaviors.

Blood collection and vaginal swabs to check for menstruation occurred at least 3x/week and on each day behavior was collected, in order to examine behavioral observations based on ovarian cycle phase. As infants, subjects were trained to separate from the group and run inside to a catch area. From the catch area, subjects were transferred to a smaller housing cage, which contained two holes from which the subject presented a leg for blood collection from the saphenous vein. Previous research has demonstrated this procedure has little influence on ovarian hormones (Blank, Gordon, & Wilson, 1983) and at the time of data collection, subjects had been exposed to this procedure for approximately two years. Vaginal swabs were collected by inserting a 6" moistened, cotton-tipped swab into the vaginal opening and were collected immediately following blood collection.

Pair-Test Procedure

Once menarche occurred, sexual behavior was examined in a pair-test (one male/one female). All lesioned females were 2.5 years of age, six control females were 2.5 years of age, and the remaining three control females were 3.5 years of age. Females were temporarily removed from their social group and tested in a separate outdoor enclosure (4.9m x 4.9m x 2.4m) that contained a perch (3.5m), which allowed the subject and males to be on different levels and to be more than 3m apart from each other. Each female was tested at least once a week, with additional tests 11-15 days after menstruation, for at least one ovarian cycle or ten weeks if regular ovarian cycles were not detected via menstruation. Pair-tests were a minimum of 30 minutes and tests were extended if the female was observed to be mounted by the male or actively soliciting the male. Each female was tested with singly with each of two unfamiliar, adult males each

day, so that the female had one test with each male every testing day. Order of males was counterbalanced throughout testing. Habituation to the testing area (minimum of 4 sessions) and to the males (2-3 sessions) occurred prior to testing in 30 minute sessions.

Group Observation Procedure

At approximately 3.5 years of age, female sexual behavior was examined in the subject's social group, with observations occurring when subjects were outdoors with indoor access prevented. Focal observations of the female subjects occurred 3x/week for approximately one hour, with more frequent observations if a female was observed initiating proximity to, following, being mounted, or soliciting the male or being mounted by the male on a nonscheduled test day. Observations typically occurred in the morning, prior to 1100 hours, with no more than 3 days in between observations.

Social Rank

At the age of the subjects (3.5yr), social rank is still determined by the mother's social rank. Each subject was assigned a rank just below that of their mother and this rank number was divided by the number of females in the group over the age of three. This allows comparing social ranks of females from groups of different sizes. Social rank was calculated for each animal using the rank at the start of the breeding season when approximately 3.5 years of age.

Hormonal Assays

Estradiol and progesterone assays, using commercially prepared radioimmunoassay kits (Siemens Healthcare, Los Angeles, CA), were completed by the Biomarkers Core Laboratory at the Yerkes National Primate Research Center. The lower sensitivity limits of the estradiol and progesterone assays were 3.00pg/ml and 0.10ng/ml

respectively (Inter-assay CV: CTL Low = 4.9% at 76 pg/mL; CTL High = 5.5% at 465 pg/mL (Estradiol), Kit C1: 14.7% at 1.58 ng/ml (n=180) , Kit C2: 13.5% at 2.91 ng/ml (n=180) , Kit C3: 11.67% at 12.01 ng/ml (n=180), C4 (Rh-P4) 8.14% at 5.40 ng/ml (n=6), C5 (Rh-P2) 20.16% at 0.32 ng/ml (n=6) (Progesterone); Intra-assay CV: 17.61% at 31.40 pg/ml (19A.211) – human female 9/16/03 (Estradiol), 8.44% (n=12 reps.) 7.73% at n=7 reps. (6-7/03) (Progesterone)).

Statistical Analyses

Hormone values for behavioral tests were determined retrospectively and were used to group already completed behavioral tests in appropriate hormonal conditions. Pair tests were grouped into three categories for analysis according to estradiol levels or menstruation dates. Luteal phase pair tests (Neo-C: 24 tests; Neo-A: 22 tests) had progesterone levels greater than 1ng/ml, when the test was within one day of a progesterone increase, or when the test occurred after the estradiol peak. All tests that were not associated with a progesterone increase or that occurred at least fourteen days prior to menstruation were grouped based on whether estradiol levels were detectable (Neo-C: 46 tests; Neo-A: 22 tests) or undetectable (Neo-C: 32 tests; Neo-A: 24 tests).

Social group observations were grouped into follicular phase or luteal phase tests for analysis. Luteal phase social group observations (Neo-C: 113 observations; Neo-A: 62 observations) were defined using the same criteria used for pair tests. Follicular phase group observations (Neo-C: 130 observations; Neo-A: 50 observations) were defined as observations that occurred after the first day of menstruation, during the rise in estradiol, until the decline in estradiol and/or 1-2 days prior to the luteal rise in progesterone.

Social group observations were not classified into whether estradiol levels were detectable or undetectable as too few observations had undetectable estradiol levels.

Behaviors analyzed for the pair tests and group observations are presented in Table 1. In the pair tests, all behaviors scored were interactions between the male and female or female self-directed behaviors. However, in the social group observations, interactions involving the focal female were not limited to adult males and could include interactions with adult females or juveniles. For social group observations, male-female interactions were analyzed, but the frequency of mounting, presents, and sexual solicits to all animals, including adult males, were also analyzed to include sexual behavior that was not directed towards an adult male. All behavioral frequencies or durations were converted into rates per hour to account for differences in observation time.

Analysis of both the pair tests and social group observations were completed using linear regression models and correlations, with each observation serving as one data point. For pair tests when estradiol levels were detectable, linear regression models, using estradiol as the first predictor and neonatal treatment (control vs. lesion) as the second predictor, were performed for each behavior. Correlations between estradiol levels and the rate of each behavior were performed to examine relationships within lesioned and control females. Neonatal treatment was used as the predictor variable in regression models to examine pair test behaviors during the luteal phase or when estradiol was undetectable. For follicular phase group observations, social rank, estradiol levels, and neonatal treatment were entered as predictor variables, in that order, in one regression model for all females for each behavior. Behaviors during luteal phase group observations were also examined using social rank and neonatal treatment as predictor

variables, in that order, for all females. Analysis within lesioned or control females was completed for follicular and luteal phase tests using linear regression or correlations respectively. Regression statistics for each predictor variable, as well as the statistics for the regression model when this variable was added, are presented. Independent t-tests were completed to compare social rank and estradiol levels between Neo-A and Neo-C females.

There were no differences in behavior between Neo-C and Neo-BC females and therefore, all control animals were combined into one group, Neo-C, for analysis.

Results

Pair Tests

Tests with Detectable Estradiol Levels

Estradiol levels, when detected, did not significantly differ between Neo-A (M=64.19±14.5 pg/ml) and Neo-C (M=72.51±12.89 pg/ml) females (Figure 2). The relationship between estradiol levels and female proximity initiation to the male was limited to control females, $r(46) = .48$, $p = .001$, as the relationship was not significant in lesioned females, $r(22) = -.23$, $p = .314$. Similar results were found with respect to the duration of proximity to the male in that greater estradiol levels significantly predicted longer proximity duration to the male, but only in control females (Neo-C: $r(46) = .36$, $p = .015$) with an inverse, but not significant relationship seen in Neo-A females ($r(22) = -.39$, $p = .070$). Neonatal treatment did not account for any additional variance, than the variance explained by estradiol levels, in the frequency of female-initiated proximity to the male, $\beta = .06$, $t(65) = 0.52$, $p = .602$, or the duration of proximity to the male, $\beta = -.04$, $t(65) = -0.35$, $p = .73$. Thus, the relationship between estradiol levels and frequency

or duration of proximity to the male differed by neonatal treatment, but there was no overall effect of neonatal treatment on these behaviors (Table 2).

Frequency of approach to within one meter of the male, like proximity, was only related to estradiol levels in control females, $r(46) = .48$, $p = .001$, and was not related to estradiol levels in lesioned females, $r(22) = -.26$, $p = .240$. In contrast to frequency of approach to within one meter of the male, duration of time spent outside of proximity, but within one meter of the male, was not significantly predicted by estradiol levels in control females, $r(46) = -.01$, $p = .944$, or lesioned females, $r(22) = -.34$, $p = .123$. Despite no effect of estradiol levels on the frequency of approach or duration of time spent within one meter of the male, there was a significant effect of neonatal treatment, after accounting for estradiol levels, with lesioned females initiating approach to within one meter of the male more frequently, $\beta = .46$, $t(65) = 4.32$, $p < .001$; $R^2 = .25$, $F(2, 67) = 10.54$, $p < .001$, and spending more time within one meter of the male, $\beta = .38$, $t(65) = 3.30$, $p = .002$; $R^2 = .16$, $F(2, 67) = 6.15$, $p = .004$, in comparison to control females (Table 2).

Estradiol levels did not significantly predict the frequency of following the male in control females, $r(46) = .19$, $p = .208$, or lesioned females, $r(22) = -.11$, $p = .633$. Similar to the frequency of following the male, the duration of time spent following the male was not predicted by estradiol levels for either neonatal treatment (Neo-C: $r(46) = .04$, $p = .774$; Neo-A: $r(22) = -.05$, $p = .812$). Though estradiol levels did not significantly predict the frequency or duration of following the male, when neonatal treatment was analyzed in addition to estradiol levels, lesioned females displayed a higher frequency, $\beta = .34$, $t(65) = 2.94$, $p = .005$; $R^2 = .12$, $F(2, 67) = 4.35$, $p = .017$, and

longer duration, $\beta = .30$, $t(65) = 2.58$, $p = ..012$; $R^2 = .09$, $F(2, 67) = 3.35$, $p = .041$, of following the male than did control females (Table 2).

Frequency of sexual solicits to the male was not significantly predicted by estradiol levels and this lack of a relationship was found in both Neo-C females, $r(46) = .26$, $p = .079$, and Neo- A females, $r(22) = -.25$, $p = .265$. In addition to estradiol levels, neonatal treatment did not account for any additional variance in the frequency of sexual solicits, $\beta = -.11$, $t(65) = -0.90$, $p = .372$; $R^2 = .06$, $F(2, 67) = 2.11$, $p = .129$ (Table 2).

Frequency of female presents to the male was not significantly predicted by estradiol in control females, $r(46) = .27$, $p = .067$, or lesioned females, $r(22) = -.13$, $p = .563$. However, neonatal treatment accounted for a significant amount of the variance in female presents, $\beta = .25$, $t(65) = 2.05$, $p = .045$, with lesioned females displaying more presents to the male than did control females (Table 2), though the regression model including estradiol levels and treatment was not statistically significant, $R^2 = .06$, $F(2, 67) = 2.09$, $p = .132$.

A significant relationship between mounting and estradiol levels was only found in control females, $r(46) = .42$, $p = .004$, as lesion females were not mounted by the male. Adding treatment to the regression model after estradiol did not significantly account for any additional variance in mount rate , $\beta = -.15$, $t(65) = -1.28$, $p = .205$ (Table 2). Intromissions and ejaculations were rarely observed and thus, were excluded from analysis.

In all females, male threats directed towards the female were not significantly predicted by estradiol levels in lesioned females, $r(22) = .23$, $p = .305$, or control females, $r(46) = -.14$, $p = .358$. Lesioned females received more threatening gestures from the

male, in comparison to control females, $\beta = .26$, $t(65) = 2.123$, $p = .038$, but despite receiving more threats by the male, lesioned females did not display more submissive gestures than control females, $\beta = -.04$, $t(65) = -0.29$, $p = .776$ (Table 2). Similar to threats by the male, submissive gestures were not related to estradiol levels in lesioned females, $r(22) = -.07$, $p = .769$, or control females, $r(46) = -.05$, $p = .746$. Self-scratching, a measure of anxiety, was displayed more by lesioned females than in control females, $\beta = .29$, $t(65) = 2.55$, $p = .013$, with estradiol levels and neonatal treatment accounting for 14% of the variation in scratching, $R^2 = .14$, $F(2, 67) = 5.13$, $p = .009$ (Table 2). In control females, there was no relationship between estradiol levels and frequency of scratching, $r(46) = -.002$, $p = .988$, whereas in lesioned females, greater estradiol levels were related to a greater frequency of scratching, $r(22) = .527$, $p = .012$. This effect in lesioned females was largely driven by two pair tests when both scratches and estradiol levels were high. Exclusion of these two data points in the analysis did not alter the significance of the regression model, but eliminated the relationship between estradiol levels and scratch frequency in lesioned females, $r(20) = -.31$, $p = .190$.

Tests with Estradiol Levels Below Detection

Neonatal treatment accounted for a significant amount of variance in all behaviors (Table 2), with the exception of mounts, which did not occur in either Neo-A or Neo-C females, and female sexual solicits (Table 2). Female sexual solicits were not predicted by neonatal treatment, $\beta = .06$, $t(53) = 0.45$, $p = .652$; $R^2 = .004$, $F(1, 54) = 0.21$, $p = .652$, but were also limited to only a few pair tests (Neo-C: 5 tests (one outlier excluded); Neo-A: 2 tests).

Luteal Phase Tests

Neonatal treatment significantly predicted the frequency of initiating proximity to the male, $\beta = .32$, $t(44) = 2.20$, $p = .033$; $R^2 = .10$, $F(1, 45) = 4.85$, $p = .033$, with lesioned females showing higher rates of proximity initiation, but neonatal treatment did not significantly predict the duration of time spent in proximity to the male (Table 2). Neonatal treatment did not significantly predict the frequency of proximity initiated by the male, $\beta = -.07$, $t(44) = -0.46$, $p = .646$; $R^2 = .005$, $F(1, 45) = 0.21$, $p = .646$. Thus, the effect of neonatal treatment on proximity initiation was not in response to reduced proximity initiation by the males. Neonatal treatment also significantly predicted the frequency, $\beta = .42$, $t(44) = 3.06$, $p = .004$; $R^2 = .18$, $F(1, 45) = 9.35$, $p = .004$, and duration, $\beta = .48$, $t(44) = 3.65$, $p = .001$; $R^2 = .23$, $F(1, 45) = 13.31$, $p = .001$, of approach to within one meter of the male, with lesioned females showing higher levels of these behaviors (Talbe 2).

Frequency, $\beta = .44$, $t(44) = 3.25$, $p = .002$; $R^2 = .19$, $F(1, 45) = 10.54$, $p = .002$, and duration, $\beta = .39$, $t(44) = 8.08$, $p = .007$; $R^2 = .16$, $F(1, 45) = 8.08$, $p = .007$, of following the male, as well as sexual solicits, $\beta = .44$, $t(43) = 3.19$, $p = .003$; $R^2 = .19$, $F(1, 43) = 10.14$, $p = .003$, were significantly predicted by neonatal treatment in that lesioned females higher levels of these behaviors during luteal phase tests (Table 2). In contrast to follow and sexual solicits, frequency rate of mounting and female presents were not significantly predicted by treatment (Table 2). Frequencies of threats by the male and female submissive behaviors were also not significantly predicted by neonatal treatment, but lesioned females continued to show higher rates of scratching, $\beta = .42$, $t(44) = 3.04$, $p = .004$; $R^2 = .17$, $F(1, 45) = 9.256$, $p = .004$ (Table 2).

Group Observations

Social rank did not differ between control and lesioned females, $t(11.67) = -0.01$, $p = .993$, but social rank was more variable in control females and equal variances were not assumed (Figure 3).

Follicular Phase Observations

Estradiol levels did not significantly differ between Neo-A ($M = 53.92 \pm 8.16$ pg/ml) and Neo-C females ($M = 47.14 \pm 4.57$ pg/ml), $t(178) = -0.76$, $p = .449$, for follicular phase observations (Figure 4). All means and regression statistics for behaviors during follicular phase group observations are presented in Table 3.

Frequency of proximity initiations, $\beta = -.18$, $t(174) = -2.44$, $p = .016$, and duration of time spent in proximity, $\beta = -.15$, $t(175) = -2.04$, $p = .043$, to adult males was predicted by neonatal treatment, after including social rank and estradiol levels as predictor variables, with Neo-C females initiating and spending more time in proximity to adult males (Table 3). In lesioned females, social rank accounted for a significant amount of variance in the duration of time spent in proximity to the male, $\beta = -.28$, $t(47) = -2.03$, $p = .048$*; $R^2 = .08$, $F(1, 48) = 4.12$, $p = .048$, but not in the frequency of proximity initiations. In contrast to lesioned females, in control females, social rank did not significantly predict the frequency or duration of proximity (Table 3). Estradiol levels did not account for any additional variance after social rank in the frequency of proximity initiations or duration of time spent in proximity in both Neo-A and Neo-C females (Table 3).

Neonatal treatment did not significantly predict any variance in the frequency to approach within one meter of the male or the duration of time spent within one meter of

an adult male (Table 3). The influence of social rank on the frequency of approach to one meter differed by neonatal treatment. Social rank significantly predicted frequency of approach to within one meter in Neo-A females, β = -.35, t(48) = -2.57, p = .013, with higher-ranking females approaching males more frequently than lower-ranking females, but no effect of social rank was found in Neo-C females, β = -.07, t(128) = -0.77, p = .441. In contrast to the frequency of approach, the duration of approach to within one meter of adult males was significantly predicted by social rank in control females, β = -.38, t(127) = -4.68, p < .001, with higher-ranking females spending more time at one meter of an adult male than lower-ranking females. However, this effect was limited to control females as social rank did not predict the duration of time at one meter of adult males in lesioned females, β = .06, t(48) = 0.40, p = .693. In addition to social rank, estradiol levels accounted for a significant amount of variance in the frequency of initiating approach to one meter of a male, but this relationship was restricted to Neo-C females, β = .39, t(127) = 4.76, p < .001, and was not found in Neo-A females, β = .03, t(47) = 0.18, p = .860. Duration of time spent within one meter of adult males was not significantly influenced by estradiol levels, after accounting for social rank, in Neo-C, β = .01, t(126) = 0.12, p = .905, or Neo-A females, β = .06, t(47) = 0.37, p = .714.

Neonatal treatment did not significantly predict the frequency or duration of following behavior and within neonatal treatment groups, social rank had no effect on the frequency or duration of following the male in Neo-A or Neo-C females (Table 3). Frequency, β = .44, t(127) = 5.58, p < .001, and duration, β = .36, t(127) = 4.37, p < .001, of following the male was only significantly predicted by estradiol levels in control

females, with higher estradiol levels related to greater frequencies and durations of follows, and no effect was found in lesioned females (Table 3).

Neonatal treatment significantly predicted the frequency of female sexual solicits directed towards adult males and to all animals, after using social rank and estradiol levels in the regression model, with control females showing more solicits to the adult male, β = -.18, $t(176) = -2.37$, $p = .019$, and to all animals, $\beta = -.23$, $t(176) = -3.12$, $p = .002$, than did lesioned females (Table 3). However, neither social rank nor estradiol levels predicted the frequency of solicits directed to the male or other animals within neonatal treatment group (Table 3).

Presentation of one's hindquarters to the adult male was not significantly predicted by neonatal treatment and was not predicted by social rank or estradiol levels in either Neo-A or Neo-C females(Table 3). However, neonatal treatment significantly predicted the frequency of presents to all animals, with lesioned females showing fewer presents to all animals than did control females, β = -.15, $t(176) = -2.04$, $p = .043$. In control females, social rank significantly predicted the frequency of presents to all animals, with lower-ranking females showing more presents to all animals than higher-ranking females, β = .25, $t(128) = 2.86$, $p = .005$, whereas in lesioned females, social rank did not predict the frequency of presents to all animals, β = -.13, $t(48) = -0.94$, $p = .354$. Estradiol levels, in addition to social rank, were not predictive of present frequency to all animals for Neo-A or Neo-C females.

Mounts received by adult males or mounts received by all animals were not significantly predicted by neonatal treatment (Table 3). In Neo-A females, social rank did not influence the frequency of mounts received from adult males or all animals,

whereas in Neo-C females, social rank predicted the frequency of mounts from all animals in that lower-ranking females received more mounts, but social rank had no effect on the frequency of mounts by adult males (Table 3). Estradiol levels did not significantly predict rates of mounting in control or lesioned females (Table 3). Ejaculations occurred too seldom to accurately examine this behavior. Intromissions were limited to only a few observations (n=19), only two of which were observations of lesioned females. Thus, neonatal treatment differences in rates of male intromissions could not be reliably interpreted.

In combination with social rank and estradiol levels, neonatal treatment significantly predicted the frequency of threats received from adult males, with lesioned females receiving more threats from adult males, $\beta = .21$, $t(176) = 2.95$, $p = .004$ (Table 3). In control females, estradiol levels predicted the frequency of threats received from the male, with more threats related to higher estradiol levels, $\beta = .23$, $t(127) = 2.68$, $p = .008$, whereas no effect of estradiol was found in lesioned females, $\beta = .08$, $t(47) = 0.63$, $p = .529$. In contrast to estradiol levels, social rank significantly predicted the frequency of threats received by the male in Neo-A females, $\beta = -.55$, $t(48) = -4.53$, $p < .001$, with higher-ranking females receiving more threats from the male than lower-ranking females, but social rank had no effect in Neo-C females, $\beta = .01$, $t(128) = 0.06$, $p = .949$. Neonatal treatment significantly predicted the frequency of submissive behaviors directed towards the male, $\beta = .20$, $t(176) = 2.69$, $p = .008$, with lesioned females showing more submissive behavior towards the male than control females. Social rank did not predict the frequency of submissive behaviors towards the male in control females, $\beta = .10$, $t(128) = 1.18$, $p = .240$, but social rank did predict the frequency of submissive behaviors

in lesioned females, with higher-ranking lesioned females showing more submission towards the male than lower-ranking lesioned females, β = -.49, $t(48)$ = -3.87, $p < .001$. After accounting for social rank, estradiol levels did not significantly predict the frequency of submissive behaviors towards the male in control or lesioned females (Table 3).

Luteal Phase Observations

All means and statistics for luteal phase observations are presented in Table 4. Frequency of female-initiated proximity to the male was not predicted neonatal treatment; however the duration of time spent in proximity, as well as the frequency and duration of time spent at one meter of the male (Table 4). Control females spent more time in proximity, β = -.16, $t(172)$ = -2.07, p = .040, more time within one meter, β = -.20, $t(171)$ = -2.72, p = .007, and initiated approach to within one meter, β = -.18, $t(171)$ = -2.42, p = .017, than did lesioned females. Higher-ranking control females also initiated approach to within one meter more frequently, $r(113)$ = -.19, p = .044, and spent more time at one meter, $r(112)$ = -.25, p = .009, or in proximity, $r(113)$ = -.21, p = .025, than lower-ranking control females. Social rank was not related to frequency or duration of proximity or approach to within one meter of adult males in lesioned females (Table 4).

Frequency and duration of following the male were not significantly predicted by neonatal treatment; however, higher-ranking lesioned females initiated follows more than lower-ranking lesioned females, $r(62)$ = -.36, p = .004, though duration of time spent following the male was not related to social rank in lesioned females (Table 4). Social rank was not related to the duration or frequency of following the male in control females (Table 4).

Sexual solicits to adult males or to all animals, as well as presents to adult males, were not significantly predicted by neonatal treatment or by social rank within lesioned and control females (Table 4). However, neonatal treatment significantly predicted presents to all animals, $\beta = .19$, $t(172) = 2.47$, $p = .015$, with lesioned females showing more presents to all animals than control females (Table 4). Social rank did not significantly predict presents to all animals for Neo-A or Neo-C females (Table 4).

Although rate of mounting by adult males was not predicted by neonatal treatment (Table 4), neonatal treatment predicted the frequency of mounts by all animals. Lesioned females were more frequently mounted by all animals than were control females, $\beta = .17$, $t(172) = 2.28$, $p = .024$. Social rank did not predict the frequency of mounts by adult males in Neo-A or Neo-C females (Table 4). However, the direction of the effect of social rank on mounts by all animals differed by neonatal treatment in that higher-ranking lesioned females were mounted more by all animals than lower-ranking lesioned females, $r(62) = -.34$, $p = .006$, whereas lower-ranking control females were mounted more by all animals than higher-ranking controls, $r(113) = .38$, $p < .001$.

Threats received by adult males were not predicted by neonatal treatment, but the influence of social rank differed between lesioned and control females (Table 4). Higher-ranking lesioned females received more threats from adult males than lower-ranking lesioned females, $r(62) = -.36$, $p = .004$, but there was no relationship between social rank and male threats in control females, $r(113) = .02$, $p = .805$. Despite no effect of neonatal treatment on the frequency of male threats, lesioned females displayed more submissive behavior towards adult males than did control females, $\beta = .19$, $t(172) = 2.56$, $p = .011$

(Table 4). In Neo-A and Neo-C females, submissive behavior directed towards adult males was not predicted by social rank (Table 4).

Due to the methodology of data collection and statistical analysis, the relationship between lesion extent and behaviors was not able to be reliably examined.

Discussion

Neonatal amygdalectomy affected female's interactions with males in a sexual context. In pair tests where there was no competition with other females, lesioned females consistently displayed higher frequencies and durations of approach to within one meter and following the male in comparison to control females, regardless of estradiol levels or ovarian cycle phase. Control females did not display higher rates of solicits or presents to the male in comparison to lesioned females during the pair tests, but rather lesioned females displayed higher rates of presents to the male when estradiol was detectable and undetectable and higher rates of solicits to the male during the luteal phase. Thus, consistent with the findings regarding distance to the male, lesions of the amygdala did not decrease female-initiated behavior in the pair tests.

Estradiol level, which affected the behavior of control females, did not predict the male-directed behavior of lesioned females as there was no significant relationship between estradiol levels and the frequency or duration of approach or following the male in lesioned females. Thus, unlike brain-intact females, where elevated estradiol increases interest in males, lesioned females appeared to show a continued interest in the male, which was not modulated by estradiol levels or ovarian cycle phase. Measures of proximity to the male during pair tests did not differ between lesioned and control females when estradiol levels were detectable, which is inconsistent with the results from

Spies and colleagues (1976) showing decreased female-initiated proximity in females following adult bilateral amygdala lesion surgery. Failure to detect differences in proximity to the male between lesioned and control females may reflect that our lesions were done neonatally, whereas Spies, et al. (1976) lesioned their females as post-pubertal adults. Although the differences may stem from a variety of procedural differences between the two studies, it is possible that the reduction in initiation of proximity in Spies, et al. (1976) study reflects changes in responsiveness to estradiol following puberty that were interrupted by the lesions. An alternate interpretation is that both our results and those of Spies and colleagues reflects a reduced sensitivity to estradiol in modulating female's male-directed behavior. In the previous study, lesioned females did not show increased initiation of proximity typical of females during the late follicular phase and in our case, male-directed behaviors were consistently elevated regardless of the amount of estradiol the female was producing. If there is a decreased sensitivity to estradiol it is interesting that it is seen in behavioral endpoints, but doesn't appear to alter HPG-axis function as lesioned females continue to have functional ovarian cycles. More detailed work on feedback mechanisms will be necessary to determine whether amygdalectomy alters estradiol sensitivity more broadly than seen in behavioral effects.

Similar to the results from Spies and colleagues (1976), the rate of mounting by males did not differ between lesioned females and control females in pair tests regardless of estradiol levels or menstrual cycle phase. The lack of a difference between lesioned and control females likely reflects the very low rates of mounting in our pair-tests. The explanation for this is not clear. It may reflect that our subjects lived in socially complex groups between pair-tests and even though we extensively habituated our subjects to the

pair-testing environment, it was unfamiliar enough to the adult males that their sexual behavior was suppressed. The males used in the Spies, et al. (1976) study exclusively mated in 10min pair-tests and thus, were highly trained for sexual activity in the pair-test context. Our finding that estradiol levels predicted control females, but not lesioned female's male-directed behavior support detecting estradiol's effects using the female's behavior instead of male's behavior directed towards the female (Zehr, et al., 1998).

Lesioned females received more threats from the male than did controls in all non-luteal phase tests, but only showed more submissive gestures than control females when estradiol was below detection. However, the overall rates of threats by the male were much lower than the rates of submissive behaviors shown by the females for both lesioned and control females. Thus, all females appeared to be overly submissive during the pair tests. It is possible that the increased number of threats by adult males to lesioned females when estradiol levels were detected or below detection resulted from lesioned females maintaining proximity and/or approach to within one meter of the male for longer durations in comparison to control females. Lastly, lesioned females consistently showed greater rates of self-scratching, a measure of anxiety, than did control females, which suggests that lesioned females were more anxious during pair tests than control females. It is not likely that this increase in anxiety in lesioned females is related to a greater number of interactions or maintaining a close distance to the male because the duration of time spent within one meter of the male was not related to the frequency of self- scratching in lesioned females (data not shown). It is also possible that the males were displaying anxiety and thus, female anxiety may have been in response to some aspect of male behavior that was not measured.

As with the pair tests, we found evidence that lesioned females may be less sensitive to estradiol in that signficiant relationships between estradiol and behavior in follicular phase group observations were only found in control females and estradiol levels were unrelated to rates of behavior in lesioned females. For example, higher frequencies and durations of approaching to one meter of the male and following the male were predicted by higher estradiol levels in control females, but were not similarly predicted in lesioned females. Male threats to control, but not lesioned females were predicted by estradiol levels, possibly reflecting the increased closeness of control females under elevated estradiol.

We predicted that in social group observations, lesioned females would show lower rates of female-initiated sexual behavior than would control females. When rates of behavior differed between lesioned and control females in the social group observations, control females showed higher rates of behavior, which is consistent with our prediction. Similar to the effects found in adult-lesioned female rhesus macaques (Spies et al., 1976) and in contrast our findings in the pair tests, control females initiated proximity more often and remained in proximity for longer periods of time than did lesioned females during follicular phase group observations. However, unlike in our pair tests, rates of initiating or remaining within one meter of the male did not differ between lesioned and control females in follicular phase group observations. This difference between proximity intiation and being within one meter of the male may reflect that males behaved differently towards lesioned females. Support for this comes from the finding that lesioned females received more threats from the males and displayed more submissive behaviors to the males than did control females. Interestingly, higher-ranking

lesioned females, who spent more time near the males, received more threats and were more submissive to adult males than were lower-ranking lesioned females. Thus, the effects of social rank on threat and submission behavior in lesioned females may be the consequence of the effects of social rank on maintaining a close distance to the male.

Despite the fact that control females spent more time in proximity to adult males than lesioned females, the frequency of being mounted by the males did not differ between lesioned and control females during follicular phase group observations. Similarly, presents to the adult male, that were not associated with his approach or contact by the male, did not differ by neonatal treatment group, which does not support the hypothesis that lesioned females would show less sexual behavior than control females. Sexual solicits, on the other hand, were displayed at higher frequencies by control females than by lesioned females. Taking all of these measures together, there is some support for the notion that neonatally lesioned females showed reduced sexual behavior, which may reflect an inherent reduction in sexual motivation (libido) or a reduced sensitivity to the sexual-motivation enhancing properties of estradiol.

Similar to the follicular phase tests and opposite the findings in the pair tests, in luteal phase tests, when neonatal treatment significantly predicted variance in proximity, approach or following behaviors, control females showed higher levels of these behaviors than lesioned females. Control females spent more time in proximity and within one meter of the male and more frequently initiated approach to one meter than lesioned females, and within control females, higher-ranking females had higher rates of these behaviors than lower-ranking females. Interestingly, these same relationships between social rank and duration of proximity or frequency of approach did not exist in control

females during follicular phase observations, which is consistent with the idea that when estradiol levels are elevated, females were more willing to be near the male, regardless of their social rank (Wallen, 1999). Thus, during the luteal phase, female attempts to be near the male are influenced by neonatal treatment and the influence of rank differs by neonatal treatment.

Mounts by adult males and solicits and presents to adult males did not differ by neonatal treatment during luteal phase observations. Thus, it is also possible that sexual behavior in lesioned females living in social groups is less coupled to the ovarian cycle in comparison to the decline in sexual behavior that is typically observe during the luteal phase in females (Wilson et al., 1982; Wallen et al., 1984).

In summary, when sexual behavior was examined in a pair test, lesioned females did not show decreased levels of female-initiated behavior and if any neonatal treatment difference existed, lesioned females showed more of that behavior than did control females. However, levels of sexual activity in the pair-tests were generally low and thus, these findings may reflect behavior that is under little modulation by sexual motivation. In contrast to the pair tests, in the social group observations, control females showed higher rates of some female-initiated sexual behaviors than did lesioned females. Despite the neonatal treatment differences in female-initiated behavior, mounting by adult males did not significantly differ between lesioned and control females in pair tests or social group observations. Though the amygdala may influence aspects of female proceptivity, it does not appear to be involved in regulating female sexual receptivity, which is consistent with data from adult-lesioned animals (Spies et al., 1976). Significant relationships between estradiol level and female-initiated sexual behaviors were limited

to control females. This supports the notion that the sexual behavior lesioned females express in their social group is less coupled to estradiol levels in comparison to control females. In conclusion, these results do not show an overall reduction in sexual behavior by lesioned females, but support the notion that they may be less sensitive to the sexual motivation enhancing effects of estradiol. Given that this is a correlational study, more mechanistic studies will be necessary to firmly identify whether differences in sensitivity to estradiol exist.

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References

Amaral, D. G., Price, J. L., Pitkanen, A., & Carmichael, S. T. (1992). Anatomical Organization of the Primate Amygdaloid Complex, In. J.P. Aggleton, (Ed.), *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction* (pp.1-66). New York: Wiley-Liss, Inc.

Bennett, A. L., Greco, B., Blasberg, M. E., & Blaustein, J. D. (2002). Response to male odours in progestin receptor- and oestrogen receptor-containing cells in female rat brain. *Journal of Neuroendocrinology*, *14*, 442-449.

Bermant, G., & Westbrook, W. H. (1966). Peripheral factors in the regulation of sexual contact by female rats. *Journal of Comparative Physiology & Psychology*, *61*, 244-250.

Butcher, R. L., Collins, W. E., & Fugo, N. W. (1974). Plasma concentrations of LH, FSH, prolactin, progesterone, and estradiol-17B throughout the 4-day estrous cycle of the rat. *Endocrinology*, *94*, 1704-1708.

Guarraci, F. A., Megroz, A. B., & Clark, A. S. (2004). Paced mating behavior in the female rat following lesions of the three regions responsive to vaginocervical stimulation. *Brain Research*, *999*, 40-52.

Goy, R. W. (1979). Sexual compatibility in rhesus monkeys: predicting sexual behavior of oppositely sexed pairs of adults. *Ciba Foundation Symposium*, *62*, 227-255.

Kling, A., & Cornell, R. (1971). Amygdalectomy and social behavior in the caged stump-tailed macaque (*M. speciosa*). *Folia Primatologica*, *14*, 91-103.

Knobil, E. (1974). On the control of gonadotropin secretion in rhesus monkeys. *Recent Progress in Hormone Research*, *30*, 1-46.

Kondo, Y., & Sakuma, Y. (2005). The medial amygdala controls the coital access of female rats: A possible involvement of emotional responsiveness. *Japanese Journal of Physiology, 55*, 345-353.

Mascó, D. H., & Carrer, H. F. (1980). Sexual receptivity in female rats after lesion or stimulation in different amygdaloid nuclei. *Physiology & Behavior, 24*, 1073-1080.

Mathews, D., Donovan, K. M., Hollingsworth, E. M., Hutson, V. B., & Overstreet, C. T. (1983). Permanent deficits in lordosis behavior in female rats with lesions of the ventromedial nucleus of the hypothalamus. *Experimental Neurology,79*, 714-719.

Mathews, D., & Edwards, D. A. (1977). Involvement of the ventromedial and anterior hypothalamic nuclei in the hormonal induction of receptivity in the female rat. *Physiology & Behavior*, *19*, 319-326.

Oomura, Y., Aou, S., Koyama, Y., Fujita, I., & Yoshimatsu, H. (1988). Central control of sexual behavior. *Brain Research Bulletin*, *20*, 863-870.

Pfaff, D. (1970). Nature of sex hormone effects on rat sex behavior: Specificity of effects and individual patterns of response. *Journal of Comparative and Physiological Psychology*, *73*(3), 349-358.

Raper, J., Bachevalier, J., Wallen, K., and Sanchez, M. (2013). Neonatal amygdala lesions alter basal cortisol levels in infant rhesus monkeys. *Psychoneuroendocrinology*, *38*(6), 818-829.

Spies, H. G., Norman, R. L., Clifton, D. K., Ochsner, A., J., Jensen, J. N., & Phoenix, C. H. (1976). Effects of bilateral amygdaloid lesions on gonadal and pituitary hormones in serum and on sexual behavior female rhesus monkeys. *Physiology &*

Behavior, *17*, 985-992.

Spiteri, T., Musatov, S., Ogawa, S., Ribeiro, A., Pfaff, D. W., & Agmo, A. (2010). The role of the estrogen receptor α in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety, and aggression. *Behavioural Brain Research*, *210*(2), 211-20.

Thompson, C., Schwartzbaum, J. S., & Harlow, H. F. (1969). Development of social fear after amygdalectomy in infant rhesus monkeys. *Physiology & Behavior*, *4*, 249-254.

Van Goozen, S. H. M., Wiegant, V. M., Endert, E., Helmond, F. A., & Van de Poll, N. E. (1997). Psychoendocrinological assessment of the menstrual cycle: The relationship between hormones, sexuality, and mood. *Archives of Sexual Behavior*, *26*, 359-382.

Wallen, K. (1990). Desire and ability: Hormones and the regulation of female sexual behavior. *Neuroscience & Biobehavioral Reviews*, *14*, 233-241.

Wallen, K. (1999). Risky business: Social context and hormonal modulation of primate sexual desire. , In. K. Wallen & J. Schneider, (Eds.), *Reproduction in Context* (pp.289-323). New York: Wiley-Liss, Inc.

Wallen, K. (2001). Sex and context: Hormones and primate sexual motivation. *Hormones & Behavior*, *40*, 339-357.

Wallen, K., Winston, L. A., Gaventa, S., Davis-DaSilva, M., & Collins, D. C. (1984). Periovulatory changes in female sexual behavior and patterns of ovarian steroid secretion in group-living rhesus monkeys. *Hormones and Behavior*, *18*, 431-450.

Wilson, M. E., & Gordon, T. P. (1989). Season determines timing of first ovulation in

rhesus monkeys (*Macaca mulatta*) housed outdoors. *J Reprod Fertil*, *86*, 435-444.

Wilson, M. E., Gordon, T. P., & Collins, D. C. (1982). Variation in ovarian steroids associated with the annual mating period in female rhesus (Macaca mulatta). *Biology of Reproduction*, *27*, 530-539.

Wilson, M. E., Gordon, T. P., & Collins, D. C. (1986). Ontogeny of luteinizing hormone secretion and first ovulation in seasonal breeding rhesus monkeys. *Endocrinology*, *118*, 293-301.

Yahr, P., & Greene, S. B. (1992). Effects of unilateral hypothalamic manipulations on the sexual behaviors of rats. *Behavioral Neuroscience*, *106*, 698-709.

Zehr, J. L., Maestripieri, D., & Wallen, K. (1998). Estradiol increases female sexual initiation independent of male responsiveness in rhesus monkeys. *Hormones and Behavior*, *33*, 95-103.

Table 1. Description of behaviors collected during behavioral observations.

Behavior	Definition
Proximity to adult male	within arm's reach of the male
Approach to within 1 meter of adult male	outside of proximity, but within 1 meter of the male
Following an adult male	persistent trailing of the male while both animals are in motion
Sexual solicits	handslap, headbob, crouch, stand, or threat-away directed towards another animal
Presents	hindquarters of the female are directed towards the recipient, does not include presents associated with an animal's approach or when another animal guides the female into a present position
Mount	mounting of the female's hindquarters
Intromission	behavioral pattern where thrusting during a mount becomes deeper and more rhythmic
Ejaculatory reflex	release of ejaculate or the behavioral pattern which characterizes it, which includes a far off gaze by the male, a continued grasp of the female, but without thrusting, and the male's legs may begin to shake
Threats by the Male	lunge, open-mouth threat, barking, or aggressive contact directed towards the female
Submission directed towards an adult male	avoidance of another animal or grimace (open mouth to show teeth while jaw is closed)
Scratch	vigorous scratching of the body, not simply brushing something off a body part (pair test only)

Table 2. Mean rates of behavior and regression model results using neonatal treatment as a predictor for behavior.

		E2 Detected	E2 Undetected	Luteal
Frequency of Female Proximity Initiation		$\beta = .06, t(65) = 0.52, p = .602$; $R^2 = .12, F(2, 67) = 4.39, p = .016$	$\beta = .47, t(54) = 3.91, p < .001$***; $R^2 = .22, F(1, 55) = 15.28, p < .001$	$\beta = .32, t(44) = 2.20, p = .033$*; $R^2 = .10, F(1, 45) = 4.85, p = .033$
Mean Frequency/ hr ± SEM	**Neo-A**	4.84 ± 1.40	11.10 ± 2.33	6.04 ± 1.58
	Neo-C	4.01 ± 1.43	2.48 ± 0.79	2.46 ± 0.57
Duration of Time in Proximity		$\beta = -.04, t(65) = -0.35, p = .730$; $R^2 = .06, F(2, 67) = 2.20, p = .119$	$\beta = .33, t(54) = 2.58, p = .013$*; $R^2 = .11, F(1, 55) = 6.64, p = .013$	$\beta = .09, t(44) = 0.58, p = .563$; $R^2 = .008, F(1, 45) = 0.34, p = .563$
Mean Duration min/hr ± SEM	**Neo-A**	1.82 ± 0.38	3.77 ± 0.85	4.10 ± 0.79
	Neo-C	2.20 ± 0.58	1.48 ± 0.44	3.49 ± 0.71
Frequency of Female Initiation within 1 meter		$\beta = .46, t(65) = 4.32, p < .001$***; $R^2 = .25, F(2, 67) = 10.54, p < .001$	$\beta = .63, t(54) = 5.90, p < .001$***; $R^2 = .39, F(1, 55) = 34.79, p < .001$	$\beta = .42, t(44) = 3.06, p = .004$**; $R^2 = .18, F(1, 45) = 9.35, p = .004$
Mean Frequency/ hr ± SEM	**Neo-A**	15.42 ± 2.39	25.45 ± 3.37	12.35 ± 2.19
	Neo-C	5.71 ± 1.14	5.85 ± 1.39	5.36 ± 0.87
Duration of Time at 1 meter		$\beta = .38, t(65) = 3.30, p = .002$**; $R^2 = .16, F(2, 67) = 6.15, p = .004$	$\beta = .51, t(54) = 4.30, p < .001$***; $R^2 = .26, F(1, 55) = 18.49, p < .001$	$\beta = .48, t(44) = 3.65, p = .001$**; $R^2 = .23, F(1, 45) = 13.31, p = .001$
Mean Duration min/hr ± SEM	**Neo-A**	4.92 ± 0.94	5.75 ± 0.70	4.86 ± 0.70
	Neo-C	2.06 ± 0.38	1.97 ± 0.55	1.81 ± 0.48
Frequency of Following the Male		$\beta = .34, t(65) = 2.94, p = .005$**; $R^2 = .12, F(2, 67) = 4.35, p = .017$	$\beta = .68, t(54) = 6.77, p < .001$***; $R^2 = .46, F(1, 55) = 45.80, p < .001$	$\beta = .44, t(44) = 3.25, p = .002$**; $R^2 = .19, F(1, 45) = 10.54, p = .002$
Mean Frequency/ hr ± SEM	**Neo-A**	8.43 ± 2.56	24.61 ± 3.83	5.73 ± 1.42
	Neo-C	2.20 ± 0.75	1.78 ± 0.56	1.15 ± 0.36
Duration of Time Following the Male		$\beta = .30, t(65) = 2.58, p = .012$*; $R^2 = .09, F(2, 67) = 3.35, p = .041$	$\beta = .67, t(54) = 6.58, p < .001$***; $R^2 = .45, F(1, 55) = 43.31, p < .001$	$\beta = .39, t(44) = 8.08, p = .007$**; $R^2 = .16, F(1, 45) = 8.08, p = .007$
Mean Duration min/hr ± SEM	**Neo-A**	0.66 ± 0.23	2.03 ± 0.34	0.35 ± 0.09
	Neo-C	0.16 ± 0.07	0.10 ± 0.04	0.08 ± 0.03

*p < .05; ** p < .01; ***p < .001. In E2 detected tests, regression model statistics represent neonatal treatment after including estradiol levels in the regression model.

Table 2 (continued)

		E2 Detected	E2 Undetected	Luteal
Mount Frequency		β = -.15, t (65) = -1.28, p = .205; R^2 = .16, F (2, 67) = 6.091, p = .004	Did not occur	β = -.001, t (44) = -0.01, p = .995; R^2 = .000, F (1, 45) = 0.00, p = .995
Mean Frequency/ hr ± SEM	**Neo-A**	0.00 ± 0.00	0.00 ± 0.00	2.06 ± 1.79
	Neo-C	2.92 ± 1.49	0.00 ± 0.00	2.07 ± 0.83
Sexual Solicit Frequency		β = -.11, t (65) = -0.90, p = .372; R^2 = .06, F (2, 67) = 2.11, p = .129	β = .06, t (53) = 0.45, p = .652; R^2 = .004, F (1, 54) = 0.21, p = .652 (1C)	β = .44, t (43) = 3.19, p = .003**; R^2 = .19, F (1, 43) = 10.14, p = .003 (1A)
Mean Frequency/ hr ± SEM	**Neo-A**	0.63 ± 0.37	1.28 ± 1.12	3.92 ± 1.29
	Neo-C	3.26 ± 1.87	0.99 ± 0.56	0.08 ± 0.08
Present Frequency		β = .25, t (65) = 2.05, p = .045*; R^2 = .06, F(2, 67) = 2.09, p = .132	β = .54, t (53) = 4.64, p < .001***; R^2 = .29, F (1, 54) = 21.56, p < .001 (1A)	β = -.07, t (42) = -0.44, p = .659; R^2 = .005, F (1, 43) = 0.20, p = .659 (1C; 1A)
Mean Frequency/ hr ± SEM	**Neo-A**	3.87 ± 1.98	6.93 ± 1.69	0.63 ± 0.20
	Neo-C	0.95 ± 0.29	0.24 ± 0.15	0.77 ± 0.24
Threats Received Frequency		β = .26, t (65) = 2.123, p = .038*; R^2 = .07, F (2, 67) = 2.26, p = .112	β = .28, t (54) = 2.12, p = .039*; R^2 = .08, F (1, 54) = 4.50, p = .039	β = -.19, t (43) = -1.24, p = .221; R^2 = .04, F (1, 44) = 1.54, p = .220 (1A)
Mean Frequency/ hr ± SEM	**Neo-A**	3.58 ± 0.93	3.09 ± 0.89	1.75 ± 0.58
	Neo-C	1.72 ± 0.41	1.07 ± 0.49	2.89 ± 0.70
Submissive Gesture Frequency		β = -.04, t (65) = -0.29, p = .776; R^2 = .004, F (2, 67) = 0.12, p = .884	β = .33, t (54) = 2.60, p = .012*; R^2 = .11, F (1, 55) = 6.74, p = .012	β = -.25, t (44) = -1.70, p = .097; R^2 = .06, F (1, 45) = 2.88, p = .097
Mean Frequency/ hr ± SEM	**Neo-A**	24.08 ± 3.68	33.17 ± 6.51	13 .96 ± 2.01
	Neo-C	25.85 ± 4.20	15.35 ± 3.41	22.25 ± 4.29
Scratch Frequency		β = .29, t (65) = 2.55, p = .013*; R^2 = .14, F (2, 67) = 5.13, p = .009	β = .51, t (54) = 4.36, p < .001***; R^2 = .26, F (1, 55) = 18.98, p < .001	β = .42, t (44) = 3.04, p = .004**; R^2 = .17, F (1, 45) = 9.256, p = .004
Mean Frequency/ hr ± SEM	**Neo-A**	8.96 ± 5.16	4.49 ± 1.02	7.91 ± 2.66
	Neo-C	0.48 ± 0.15	0.43 ± 0.26	0.17 ± 0.12

*p < .05; ** p < .01; ***p < .001. Number and treatment of outliers excluded are indicated in parentheses. In E2 detected tests, regression model statistics represent neonatal treatment after including estradiol levels in the regression model.

Table 3. Means and regression statistics for each behavior with respect to neonatal treatment group differences and within group effects of social rank and estradiol levels on social group behavior during the follicular phase.

		Neonatal Treatment	
Behavior		**Neo-A**	**Neo-C**
Proximity Initiation to Adult Male (1C; 1A)	Neonatal Treatment Effect	$\beta = -.18, t(174) = -2.44, p = .016$*; $R^2 = .04, F(3, 177) = 2.69, p = .048$	
	Mean Frequency Prox/hr ± SEM	2.49 ± 0.55	4.95 ± 0.59
	Social Rank	$\beta = -.20, t(47) = -1.39, p = .171$; $R^2 = .04, F(1, 48) = 1.94, p = .171$	$\beta = .12, t(127) = 1.30, p = .195$; $R^2 = .01, F(1, 128) = 1.70, p = .195$
	Estradiol	$\beta = -.07, t(46) = -0.44, p = .662$; $R^2 = .04, F(2, 48) = 1.05, p = .359$	$\beta = .08, t(126) = 0.91, p = .366$; $R^2 = .02, F(2, 128) = 1.26, p = .287$
Proximity to Adult Male (Duration) (1A)	Neonatal Treatment Effect	$\beta = -.15, t(175) = -2.04, p = .043$*; $R^2 = .05, F(3, 178) = 3.06, p = .030$	
	Mean Duration Prox min/hr ± SEM	0.95 ± 0.26	2.80 ± 0.56
	Social Rank	$\beta = -.28, t(47) = -2.03, p = .048$*; $R^2 = .08, F(1, 48) = 4.12, p = .048$	$\beta = -.15, t(128) = -1.68, p = .096$; $R^2 = .02, F(1, 129) = 2.82, p = .096$
	Estradiol	$\beta = .13, t(46) = 0.89, p = .376$; $R^2 = .10, F(2, 48) = 2.45, p = .097$	$\beta = -.10, t(127) = -1.19, p = .238$; $R^2 = .03, F(2, 129) = 2.12, p = .125$
Initiation at 1 meter of Adult Male	Neonatal Treatment Effect	$\beta = -.03, t(176) = -0.35, p = .731$; $R^2 = .11, F(3, 179) = 7.00, p < .001$	
	Mean Frequency at 1 meter/hr ± SEM	3.13 ± 0.66	3.18 ± 0.45
	Social Rank	$\beta = -.35, t(48) = -2.57, p = .013$*; $R^2 = .12, F(1, 49) = 6.61, p = .013$	$\beta = -.07, t(128) = -0.77, p = .441$; $R^2 = .005, F(1, 129) = 0.60, p = .441$
	Estradiol	$\beta = .03, t(47) = 0.18, p = .860$; $R^2 = .12, F(2, 49) = 3.25, p = .048$	$\beta = .39, t(127) = 4.76, p < .001$***; $R^2 = .16, F(2, 129) = 11.66, p < .001$
At 1 meter of Adult Male (Duration) (1C)	Neonatal Treatment Effect	$\beta = -.07, t(175) = -0.92, p = .357$; $R^2 = .12, F(3, 178) = 7.69, p < .001$	
	Mean Duration at 1 meter min/hr ± SEM	1.34 ± 0.30	1.68 ± 0.28
	Social Rank	$\beta = .06, t(48) = 0.40, p = .693$; $R^2 = .003, F(1, 49) = 0.16, p = .693$	$\beta = -.38, t(127) = -4.68, p < .001$***; $R^2 = .15, F(1, 128) = 21.88, p < .001$
	Estradiol	$\beta = .06, t(47) = 0.37, p = .714$; $R^2 = .006, F(2, 49) = 0.15, p = .865$	$\beta = .01, t(126) = 0.12, p = .905$; $R^2 = .15, F(2, 128) = 10.86, p < .001$
Following the Male	Neonatal Treatment Effect	$\beta = -.13, t(176) = -1.83, p = .068$; $R^2 = .15, F(3, 179) = 10.56, p < .001$	
	Mean Frequency Follow/hr ± SEM	1.25 ± 0.38	2.69 ± 0.60
	Social Rank	$\beta = -.24, t(48) = -1.69, p = .097$; $R^2 = .06, F(1, 49) = 2.87, p = .097$	$\beta = .04, t(128) = 0.49, p = .624$; $R^2 = .002, F(1, 129) = 0.24, p = .624$
	Estradiol	$\beta = .08, t(47) = 0.54, p = .595$; $R^2 = .06, F(2, 49) = 1.56, p = .221$	$\beta = .44, t(127) = 5.58, p < .001$***; $R^2 = .20, F(2, 129) = 15.72, p < .001$

*p < .05; ** p < .01; ***p < .001. Number in parentheses indicates number of outliers removed from analysis. R^2 and F-test values are presented for the regression model when each predictor was added. Neonatal treatment was added to the model following social rank and estradiol levels.

Table 3 (continued)

Behavior		Neonatal Treatment	
		Neo-A	Neo-C
Following the Male (Duration)	Neonatal Treatment Effect	$\beta = -.14, t(176) = -1.94, p = .054; R^2 = .10, F(3, 179) = 6.81, p < .001$	
	Mean Duration Follow min/hr ± SEM	0.12 ± 0.04	0.31 ± 0.07
	Social Rank	$\beta = -.09, t(48) = -0.60, p = .553;$ $R^2 = .007, F(1, 49) = 0.36, p = .553$	$\beta = .03, t(128) = 0.30, p = .762;$ $R^2 = .001, F(1, 129) = 0.09, p = .762$
	Estradiol	$\beta = .03, t(47) = 0.21, p = .836;$ $R^2 = .008, F(2, 49) = 0.20, p = .822$	$\beta = .36, t(127) = 4.37, p < .001$***; $R^2 = .13, F(2, 129) = 9.60, p < .001$
Mounts by Adult Male (2C)	Neonatal Treatment Effect	$\beta = -.02, t(174) = -0.22, p = .830; R^2 = .10, F(3, 177) = 0.42, p = .737$	
	Mean Frequency Mounts by Adult Male/hr ± SEM	0.99 ± 0.68	1.19 ± 0.32
	Social Rank	$\beta = .15, t(48) = 1.01, p = .316;$ $R^2 = .02, F(1, 49) = 1.03, p = .316$	$\beta = .04, t(126) = 0.39, p = .695;$ $R^2 = .001, F(1, 127) = 0.16, p = .695$
	Estradiol	$\beta = -.02, t(47) = -0.15, p = .879;$ $R^2 = .02, F(2, 49) = 0.52, p = .601$	$\beta = -.07, t(125) = -0.79, p = .433;$ $R^2 = .01, F(2, 127) = 0.39, p = .681$
Mounts by All Animals (2C)	Neonatal Treatment Effect	$\beta = -.04, t(174) = -0.51, p = .609; R^2 = .05, F(3, 177) = 3.19, p = .025$	
	Mean Frequency Mounts by All/hr ± SEM	2.68 ± 0.84	3.28 ± 0.50
	Social Rank	$\beta = -.12, t(48) = -0.85, p = .399;$ $R^2 = .02, F(1, 49) = 0.72, p = .399$	$\beta = .30, t(126) = 3.46, p = .001$**; $R^2 = .09, F(1, 127) = 11.98, p = .001$
	Estradiol	$\beta = .03, t(47) = 0.17, p = .864;$ $R^2 = .02, F(2, 49) = 0.37, p = .693$	$\beta = -.07, t(125) = -0.76, p = .448;$ $R^2 = .09, F(2, 127) = 6.26, p = .003$
Sexual Solicit to Adult Male	Neonatal Treatment Effect	$\beta = -.18, t(176) = -2.37, p = .019$*; $R^2 = .04, F(3, 179) = 2.47, p = .064$	
	Mean Frequency Solicit to Adult Male/hr ± SEM	1.37 ± 0.67	6.37 ± 1.33
	Social Rank	$\beta = .14, t(48) = 0.98, p = .334;$ $R^2 = .02, F(1, 49) = 0.95, p = .334$	$\beta = -.04, t(128) = -0.41, p = .682;$ $R^2 = .001, F(1, 129) = 0.17, p = .682$
	Estradiol	$\beta = -.02, t(47) = -0.12, p = .909;$ $R^2 = .02, F(2, 49) = 0.47, p = .627$	$\beta = .14, t(127) = 1.60, p = .112;$ $R^2 = .02, F(2, 129) = 1.37, p = .258$
Sexual Solicits to All Animals	Neonatal Treatment Effect	$\beta = -.23, t(176) = -3.12, p = .002$**; $R^2 = .08, F(3, 179) = 4.90, p = .003$	
	Mean Frequency Solicit to All/hr ± SEM	1.47 ± 0.67	8.60 ± 1.42
	Social Rank	$\beta = .14, t(48) = 0.98, p = .331;$ $R^2 = .02, F(1, 49) = 0.97, p = .331$	$\beta = .12, t(128) = 1.33, p = .184;$ $R^2 = .01, F(1, 129) = 1.78, p = .184$
	Estradiol	$\beta = -.03, t(47) = -0.19, p = .851;$ $R^2 = .02, F(2, 49) = 0.49, p = .615$	$\beta = .15, t(127) = 1.72, p = .087;$ $R^2 = .04, F(2, 129) = 2.39, p = .096$

*p < .05; ** p < .01; ***p < .001. Number in parentheses indicates number of outliers removed from analysis. R^2 and F-test values are presented for the regression model when each predictor was added. Neonatal treatment was added to the model following social rank and estradiol levels.

Table 3 (continued)

Behavior		Neonatal Treatment	
		Neo-A	Neo-C
Present to Adult Male	Neonatal Treatment Effect	β = -.08, t (176) = -1.10, p = .271; R^2 = .01, F (3, 179) = 0.60, p = .614	
	Mean Frequency Present to Adult Male/hr ± SEM	0.69 ± 0.33	1.26 ± 0.31
	Social Rank	β = -.09, t (48) = -0.62, p = .540; R^2 = .01, F (1, 49) = 0.38, p = .540	β = -.04, t (128) = -0.49, p = .625; R^2 = .002, F (1, 129) = 0.24, p = .625
	Estradiol	β = -.08, t (47) = -0.50, p = .623; R^2 = .01, F (2, 49) = 0.31, p = .735	β = .07, t (127) = 0.77, p = .441; R^2 = .007, F (2, 129) = 0.42, p = .659
Present to All Animals	Neonatal Treatment Effect	β = -.15, t (176) = -2.04, p = .043*; R^2 = .07, F (3, 179) = 4.536, p = .004	
	Mean Frequency Present to All/hr ± SEM	1.15 ± 0.38	2.64 ± 0.43
	Social Rank	β = -.13, t (48) = -0.94, p = .354; R^2 = .02, F (1, 49) = 0.88, p = .354	β = .25, t (128) = 2.86, p = .005**; R^2 = .06, F (1, 129) =8.19, p = .005
	Estradiol	β = -.06, t (47) = -0.37, p = .715; R^2 = .02, F (2, 49) = 0.50, p = .611	β = .07, t (127) = 0.75, p = .453; R^2 = .06, F (2, 129) = 4.36, p = .015
Threats Received by Adult Male	Neonatal Treatment Effect	β = .21, t (176) = 2.95, p = .004**; R^2 = .10, F (3, 179) = 6.23, p < .001	
	Mean Frequency Threats from Males/hr ± SEM	2.01 ± 0.75	0.53 ± 0.09
	Social Rank	β = -.55, t (48) = -4.53, p < .001***; R^2 = .30, F (1, 49) = 20.53, p < .001	β = .01, t (128) = 0.06, p = .949; R^2 = .00, F (1, 129) = 0.004, p = .949
	Estradiol	β = .08, t (47) = 0.63, p = .529; R^2 = .31, F (2, 49) = 10.34, p < .001	β = .23, t (127) = 2.68, p = .008**; R^2 = .05, F (2, 129) = 3.60, p = .030
Submissive Behaviors to Adult Male	Neonatal Treatment Effect	β = .20, t (176) = 2.69, p = .008**; R^2 = .08, F (3, 179) = 4.98, p = .002	
	Mean Frequency Female Submissive Behaviors/hr ± SEM	3.73 ± 0.83	2.03 ± 0.23
	Social Rank	β = -.49, t (48) = -3.87, p < .001***; R^2 = .24, F (1, 49) = 14.98, p < .001	β = .10, t (128) = 1.18, p = .240; R^2 = .01, F (1, 129) = 1.40, p = .240
	Estradiol	β = .12, t (47) = 0.94, p = .354; R^2 = .25, F (2, 49) = 7.91, p = .001	β = .17, t (127) = 1.91, p = .058; R^2 = .04, F (2, 129) = 2.54, p = .083

*p < .05; ** p < .01; ***p < .001. Number in parentheses indicates number of outliers removed from analysis. R^2 and F-test values are presented for the regression model when each predictor was added. Neonatal treatment was added to the model following social rank and estradiol levels.

Table 4. Mean, regression, and correlation statistics for each behavior with respect to neonatal treatment group and social rank within groups during luteal phase social group observations.

		Neonatal Treatment	
Behavior		**Neo-A**	**Neo-C**
Proximity Initiation to Adult Male (1C)	Neonatal Treatment Effect	$\beta = -.04, t(171) = -0.48, p = .634; R^2 = .01, F(2, 173) = 0.79, p = .457$	
	Mean Frequency Prox/hr ± SEM	1.32 ± 0.35	1.44 ± 0.25
	Social Rank	$r(62) = -.02, p = .907$	$r(112) = -.12, p = .212$
Proximity to Adult Male (Duration)	Neonatal Treatment Effect	$\beta = -.16, t(172) = -2.07, p = .040^*; R^2 = .06, F(2, 174) = 5.05, p = .007$	
	Mean Duration Prox min/hr ± SEM	0.67 ± 0.16	1.92 ± 0.57
	Social Rank	$r(62) = -.06, p = .667$	$r(113) = -.21, p = .025^*$
Initiation at 1 meter of Adult Male (1C)	Neonatal Treatment Effect	$\beta = -.18, t(171) = -2.42, p = .017^*; R^2 = .06, F(2, 173) = 5.03, p = .008$	
	Mean Frequency at 1 meter/hr ± SEM	0.71 ± 0.18	1.40 ± 0.23
	Social Rank	$r(62) = -.11, p = .380$	$r(112) = -.19, p = .044^*$
At 1 meter of Adult Male (Duration) (1C)	Neonatal Treatment Effect	$\beta = -.20, t(171) = -2.72, p = .007^{**}; R^2 = .08, F(2, 173) = 7.69, p = .001$	
	Mean Duration at 1 meter min/hr ± SEM	0.75 ± 0.22	1.73 ± 0.32
	Social Rank	$r(62) = -.11, p = .380$	$r(112) = -.25, p = .009^{**}$
Following the Male	Neonatal Treatment Effect	$\beta = -.11, t(172) = -1.49, p = .137; R^2 = .03, F(2, 174) = 2.69, p = .071$	
	Mean Frequency Follow/hr ± SEM	0.41 ± 0.16	1.40 ± 0.41
	Social Rank	$r(62) = -.36, p = .004^*$	$r(113) = .14, p = .133$
Following the Male (Duration) (1C)	Neonatal Treatment Effect	$\beta = -.13, t(171) = -1.72, p = .087; R^2 = .03, F(2, 173) = 2.64, p = .074$	
	Mean Duration Follow min/hr ± SEM	0.05 ± 0.02	0.22 ± 0.07
	Social Rank	$r(62) = -.25, p = .053$	$r(112) = .11, p = .240$

*p < .05; ** p < .01; ***p < .001. Number in parentheses indicates number of outliers removed from analysis. R^2 and F-test values are presented for the regression model when each predictor was added. Neonatal treatment was added to the model following social rank and estradiol levels.

Table 4 (continued)

Behavior		Neonatal Treatment	
		Neo-A	Neo-C
Mounts by Adult Male	Neonatal Treatment Effect	$\beta = -.05, t(172) = -0.63, p = .527; R^2 = .007, F(2, 174) = 0.61, p = .545$	
	Mean Frequency Mounts by Adult Male/hr ± SEM	0.13 ± 0.05	0.22 ± 0.09
	Social Rank	$r(62) = .09, p = .504$	$r(113) = .06, p = .544$
Mounts by All Animals	Neonatal Treatment Effect	$\beta = .17, t(172) = 2.28, p = .024^*; R^2 = .06, F(2, 174) = 5.69, p = .004$	
	Mean Frequency Mounts by All/hr ± SEM	2.75 ± 0.53	1.70 ± 0.32
	Social Rank	$r(62) = -.34, p = .006^*$	$r(113) = .38, p < .001^*$
Sexual Solicit to Adult Male	Neonatal Treatment Effect	$\beta = -.09, t(172) = -1.22, p = .224; R^2 = .01, F(2, 174) = 0.87, p = .419$	
	Mean Frequency Solicit to Adult Male/hr ± SEM	0.47 ± 0.26	1.76 ± 0.73
	Social Rank	$r(62) = .12, p = .343$	$r(113) = .02, p = .852$
Sexual Solicits to All Animals	Neonatal Treatment Effect	$\beta = -.09, t(172) = -1.22, p = .224; R^2 = .02, F(2, 174) = 1.91, p = .151$	
	Mean Frequency Solicit to All/hr ± SEM	0.82 ± 0.33	2.50 ± 0.84
	Social Rank	$r(62) = .12, p = .342$	$r(113) = .10, p = .302$
Present to Adult Male	Neonatal Treatment Effect	$\beta = -.07, t(172) = -0.95, p = .343; R^2 = .01, F(2, 174) = 0.97, p = .382$	
	Mean Frequency Present to Adult Male/hr ± SEM	0.26 ± 0.08	0.36 ± 0.09
	Social Rank	$r(62) = -.05, p = .718$	$r(113) = -.10, p = .317$
Present to All Animals	Neonatal Treatment Effect	$\beta = .19, t(172) = 2.47, p = .015^*; R^2 = .04, F(3, 179) = 3.45, p = .034$	
	Mean Frequency Present to All/hr ± SEM	1.73 ± 0.36	0.90 ± 0.18
	Social Rank	$r(62) = -.08, p = .531$	$r(113) = .18, p = .063$

*p < .05; ** p < .01; ***p < .001. Number in parentheses indicates number of outliers removed from analysis. R^2 and F-test values are presented for the regression model when each predictor was added. Neonatal treatment was added to the model following social rank and estradiol levels.

Table 4 (continued)

Behavior		Neonatal Treatment	
		Neo-A	**Neo-C**
Threats Received by Adult Male	Neonatal Treatment Effect	$\beta = .07, t(172) = 0.89, p = .377; R^2 = .10, F(2, 174) = 0.72, p = .487$	
	Mean Frequency Threats from Males/hr ± SEM	0.77 ± 0.24	0.51 ± 0.13
	Social Rank	$r(62) = -.36, p = .004$*	$r(113) = .02, p = .805$
Submissive Behaviors to Adult Male	Neonatal Treatment Effect	$\beta = .19, t(172) = 2.56, p = .011$*; $R^2 = .04, F(2, 174) = 3.39, p = .036$	
	Mean Frequency Female Submissive Behaviors/hr ± SEM	2.37 ± 0.39	1.33 ± 0.21
	Social Rank	$r(62) = -.19, p = .140$	$r(113) = .05, p = .614$

*p < .05; ** p < .01; ***p < .001. Number in parentheses indicates number of outliers removed from analysis. R^2 and F-test values are presented for the regression model when each predictor was added. Neonatal treatment was added to the model following social rank and estradiol levels.

Figure Captions

Figure 1. T1-Weighted MRI from a Neo-C female and the post-surgical FLAIR image from a Neo-A female, with black arrows indicating the hypersignals from edema on the FLAIR image.

Figure 2. Estradiol levels (pg/ml) for pair tests when estradiol levels were detectable for Neo-A (*n*=22) and Neo-C (*n*=46) females.

Figure 3. Social rank at the start of the breeding season when subjects were 3.5 years of age.

Figure 4. Estradiol levels during follicular phase group observations at 3.5 years of age for Neo-A observations (*n*=50) and Neo-C observations (*n*=130).

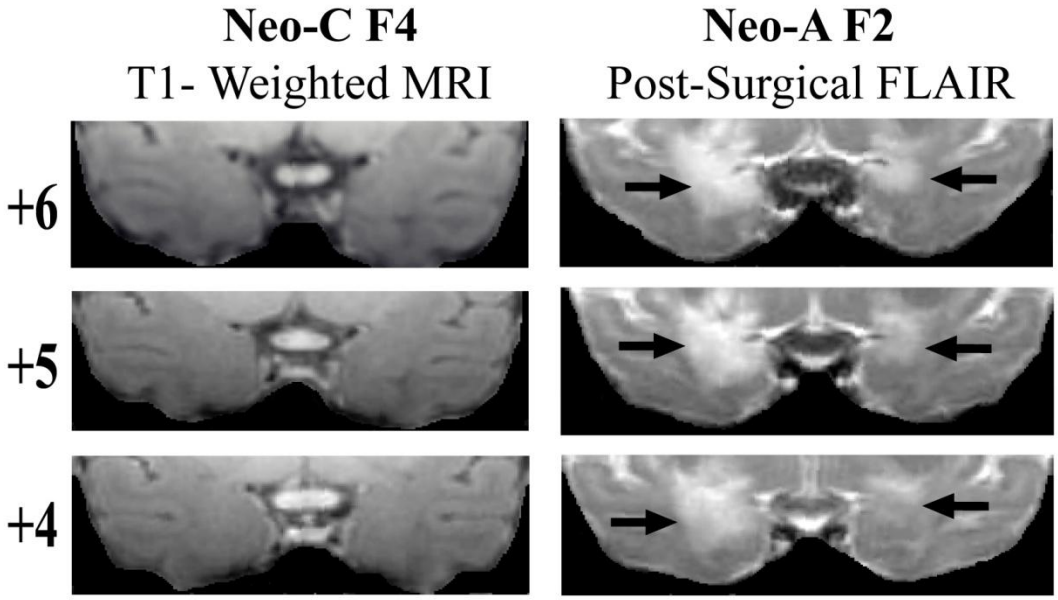

Figure 1.

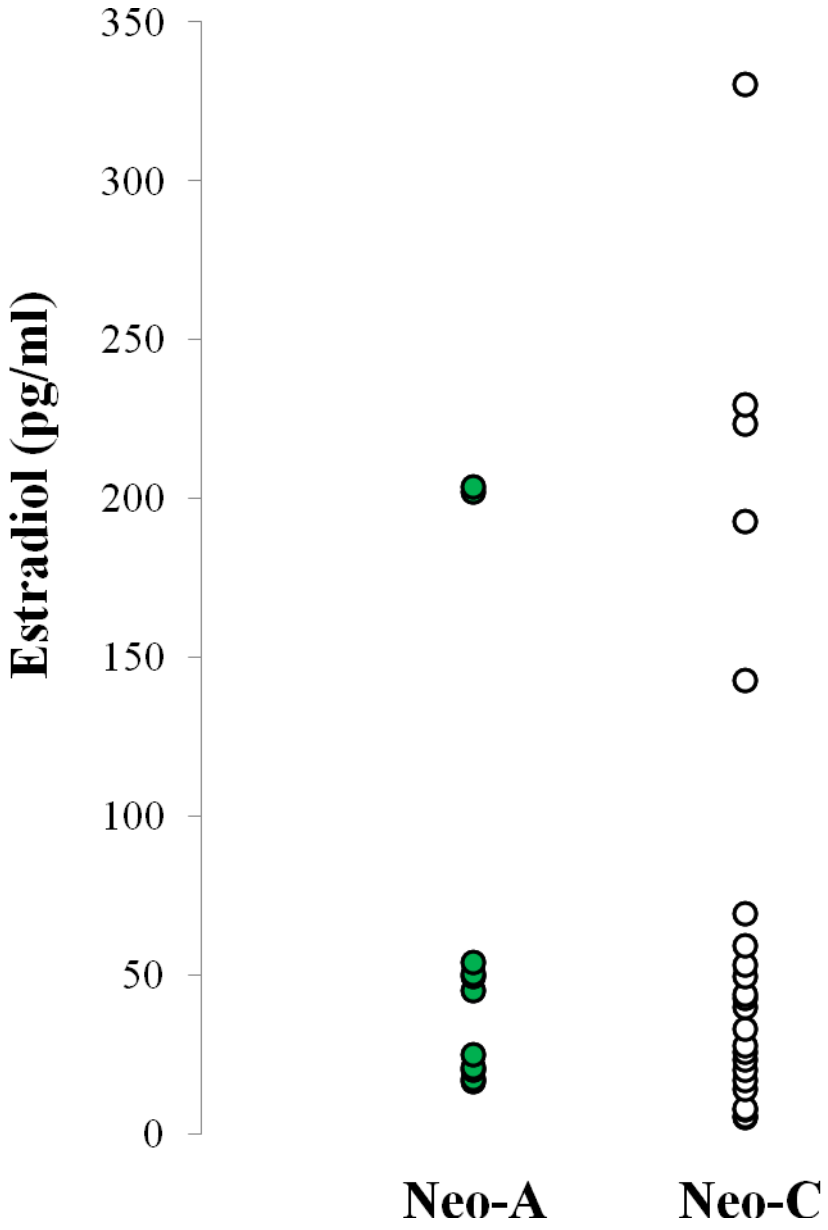

Figure 2.

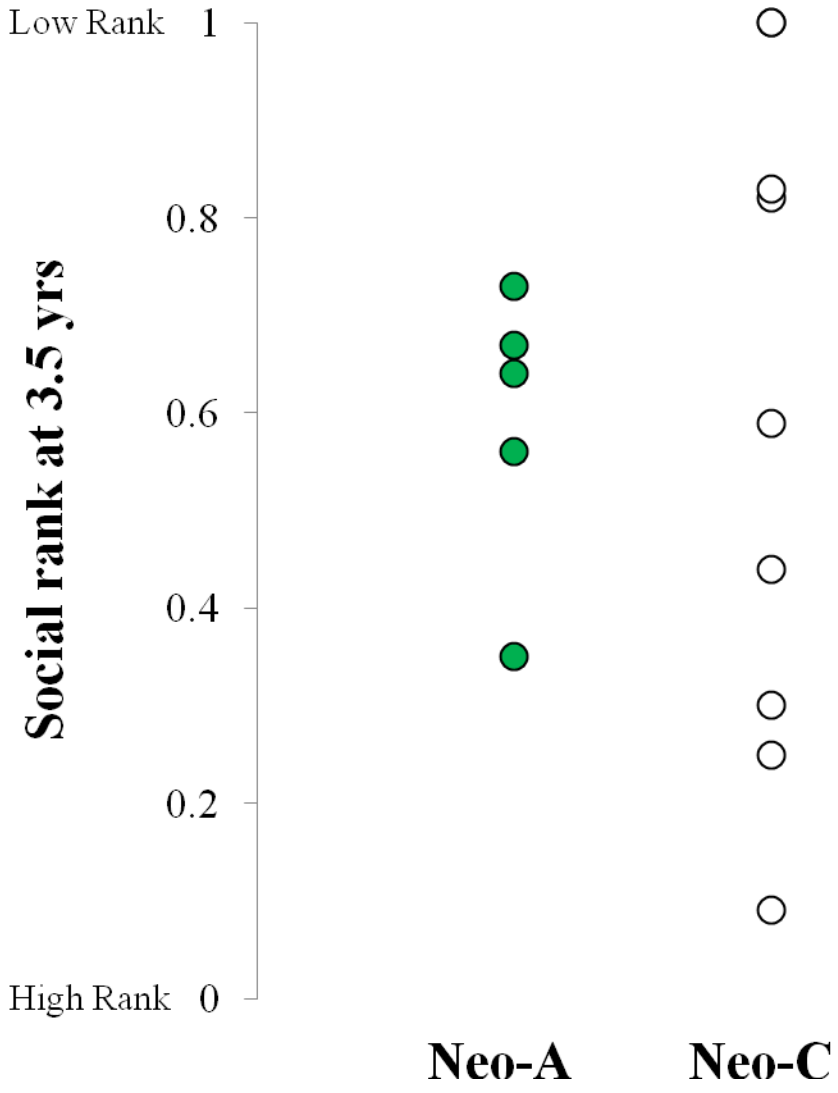
Low Rank
1
0.8
0.6
0.4
0.2
High Rank
0
Social rank at 3.5 yrs
Neo-A
Neo-C

Figure 3.

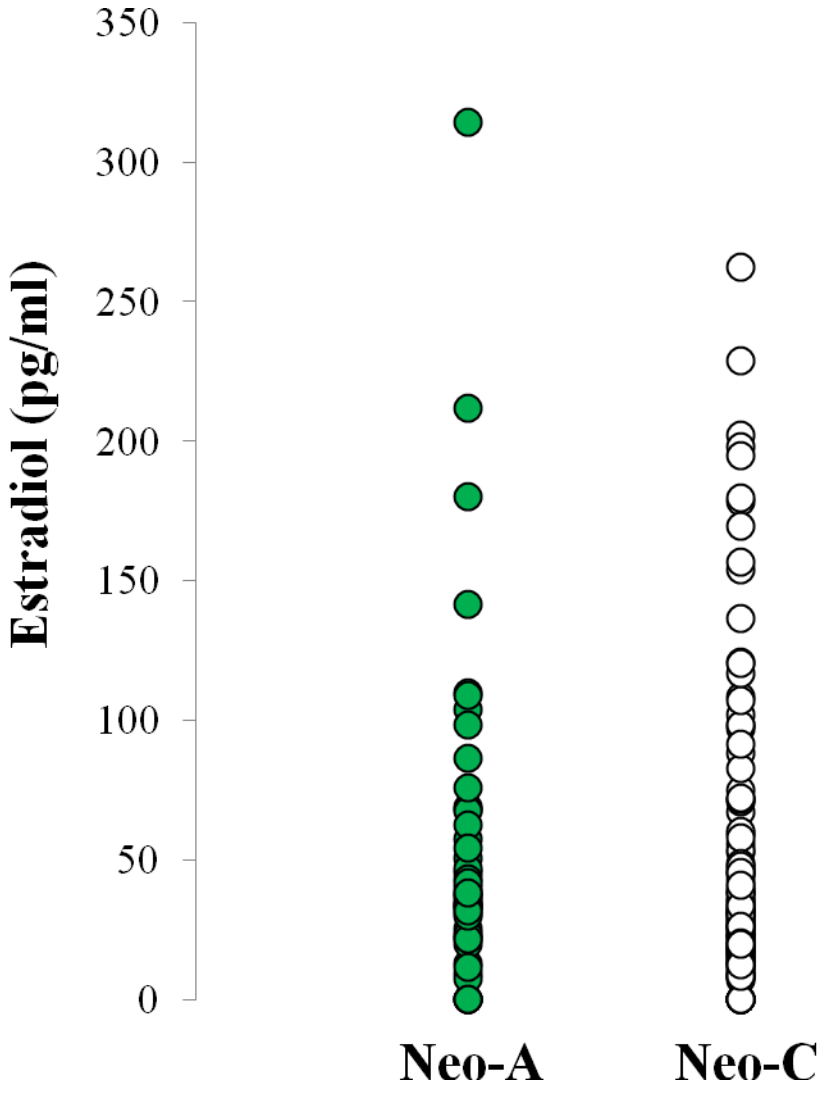

Figure 4.

General Discussion

The purpose of the current study was to examine the effects of neonatal bilateral amygdala lesions on pubertal timing and sexual behavior in female rhesus macaques to better understand the influence of the amygdala in modulating social cues that alter pubertal timing and sexual behavior.

In female rhesus macaques, menarche is an indicator of puberty onset, with the pubertal period culminating in first ovulation and adult reproductive function (Foster, 1977; Resko et al., 1982). The effects of social rank on age at menarche are not well-defined as some research suggests higher-ranking females experience earlier menarche (Wilson et al., 2013), whereas other data indicates social rank has no effect on age at menarche (Zehr et al., 2005; Wilson & Kinkead, 2008). Although it is not clear that social rank influences puberty onset, social rank consistently affects the timing of first ovulation in that lower-ranking females show less variation in the age at first ovulation and longer intervals between menarche and first ovulation than higher-ranking females (Zehr et al., 2005; Wilson & Kinkead, 2008).

Social rank also influences the expression of sexual behavior in female rhesus macaques in that higher-ranking females initiate sexual behavior more frequently than lower-ranking females when estradiol levels are increasing, with lower-ranking females engaging in behavior only around the time of the estradiol peak (Wallen, 1990). Thus, social rank influences not only the expression of behavior, but also the relationship between estradiol levels and behavior, with lower-ranking females showing behavior that is tightly coupled to estradiol levels (Wallen, 1990).

The effects of social context on sexual behavior are not limited to social rank as social group structure also modulates the expression of sexual behavior and the relationship between estradiol levels and behavior (Wallen, 1990; 2001). In multi-female groups containing one male, sexual behavior was limited to the time around the estradiol peak, demonstrating a tight coupling of estradiol levels and behavior (Wallen et al., 1984). However, in large social groups containing multiples males and females, high levels of sexual behavior were observed throughout the first half of the menstrual cycle, when estradiol levels are low, but increasing, demonstrating less of a relationship between estradiol levels and behavior (Wilson et al., 1982). In both types of social groups, the expression of sexual behavior was low during the luteal phase of the menstrual cycles, demonstrating a cyclic nature of sexual behavior related to the female's ovarian cycle (Wilson et al., 1982; Wallen et al., 1984). In contrast to the cyclic expression of the sexual behavior observed in social groups, sexual behavior during tests with one female and one male was consistent throughout the menstrual cycle (Goy, 1979), but at lower levels than those seen in the social groups (Wallen, 2001) . Thus, the social context in which behavior is observed, like social rank, influences the expression of female sexual behavior as well as the relationship between estradiol levels and female sexual behavior.

Though it is clear that social context influences pubertal timing and sexual behavior, the brain mechanism(s) modulating pubertal timing and sexual behavior based on social context are unknown. The amygdala is one potential region of the brain that modulates the effects of social context on pubertal timing and sexual behavior. The amygdala is important for aspects of social behavior including social recognition,

development of opposite-sex preferences, and responding to social cues appropriately (Thompson, Schwartzbaum, & Harlow, 1969; Kling & Cornell, 1971; Petrulis & Johnston, 1999; Bennett, Greco, Blasberg, & Blaustein, 2002; Spiteri, Musatov, Ogawa, Ribeiro, Pfaff, & Agmo, 2010). The amygdala also projects to regions of the hypothalamus important for neuroendocrine control (as reviewed by Amaral, Price, Pitkänen, & Carmichael, 1992) and thus, the amygdala has the potential to influence pubertal timing and sexual behavior.

The current study examined the effects of neonatal bilateral amygdala lesions on pubertal timing and sexual behavior in females living in social groups. The specific aims of this study addressed whether the amygdala influences pubertal timing, if lesions of the amygdala eliminated the effects of social rank on pubertal timing, and how neonatal amygdala lesions altered sexual behavior in different social contexts. We predicted that lesions of the amygdala would result in earlier age at menarche, if social rank influenced age at menarche, and would reduce the variation in age at first ovulation because the effects of social rank on age at first ovulation would be eliminated. We predicted that lesioned females would show lower levels of sexual behavior than control females and that these differences in behavior would be more pronounced in the social group than during pair tests.

Does the amygdala influence the timing of puberty?

In the present study, neonatal lesions of the amygdala resulted in earlier menarche and first ovulation in comparison to control females, indicating that puberty onset was advanced in lesioned females. This finding is inconsistent with previous research showing that lesions of the amygdala at 10-13 months of age had no effect on age at

menarche or first ovulation (Norman & Spies, 1981). This difference in findings between studies may result from differences in the age at which the lesion occurred. In the current study, lesions of the amygdala occurred at approximately one month of age, when the amygdala is increasing in volume (Payne et al., 2010), whereas lesions of the amygdala at 10-13 months of age in the previous study occurred after the amygdala has developed (Payne et al., 2010). In rats, the effects of amygdala lesions on pubertal timing were dependent on the age at which the lesion occurred (Döcke, 1974; Docke et al., 1976; Döcke et al., 1980) and thus, it is possible that earlier menarche and first ovulation in lesioned females in the current study was a result of the timing of the lesion.

Data in both girls and female rhesus macaques has shown that greater body weights were related to earlier age at menarche (Moffit et al., 1992; Terasawa et al., 2012); however, earlier menarche in all lesioned females was not the result of increased body weight at the time of menarche. Body weight during the breeding season at 1.5 years of age, when all lesioned females experienced menarche, was not statistically different between lesioned females and control females that reached menarche one year later. In fact, there was a statistical trend for lower body weights at 1.5 years of age in lesioned females in comparison to body weights at 1.5 years of age for control females that experienced menarche a year later, which would likely have been significant with larger sample sizes. Body weights at 1.5 years of age did not differ between lesioned females and control females that reached menarche at 1.5 years of age, indicating that lower body weights at 1.5 years of age were characteristic of all females reaching menarche at 1.5 years of age, regardless of the presence or absence of the amygdala. Thus, earlier menarche in all lesioned females is not the result of greater body weights at

1.5 years of age. Social rank and extent of amygdala lesion in the right hemisphere were negatively related to each other and both were related to age at menarche, with earlier age at menarche related to higher social rank and greater damage in the right amygdala. It is possible that one or both of these factors has an effect independent of the other on age at menarche and could explain the earlier age at menarche in lesioned females. However, due to the relationship between social rank and right amygdala damage, it is not possible to determine how each factor may influence age at menarche.

Similar to menarche, regression analysis indicated first ovulation occurred earlier in lesioned females in comparison to control females. Because menarche occurred earlier in these females, it is possible that earlier first ovulation is simply a consequence of earlier menarche, and that lesions of the amygdala do not specifically influence age at first ovulation. If the amygdala had an effect on age at first ovulation that was independent of its influence on age at menarche, then duration of adolescent sterility should be shorter in lesioned females. Duration of adolescent sterility did not differ between lesioned and control females, which suggests that the influence of the amygdala is limited to puberty onset. Although earlier age at first ovulation was related to sparing of the left amygdala, the meaning of this relationship is not clear. Unilateral lesions of the left medial amygdala in adult female rats prolonged the estrous period (Sanchez & Dominguez, 1995), which is consistent with the finding that greater damage in the left hemisphere was related to a later age at first ovulation. However, the study in rodents was completed in adult females, with lesions of the amygdala occurring in adulthood, lesions were specific to the medial amygdala, and the lesions were unilateral (Sanchez & Dominguez, 1995). Thus, there are several important differences between the current

study and the study in rodents and the consistency of these findings should be interpreted cautiously. Alternatively, the relationship between left lesion damage and age at first ovulation may not be meaningful as adolescent sterility did not differ between lesioned and control females. The factors influencing the variation in age at first ovulation were similarly present in both lesioned and control females, despite the amygdala damage in lesioned females, making the relationship between left amygdala damage and age at first ovulation difficult to interpret.

Age at first ovulation was more variable in lesioned females, spanning across three breeding seasons, whereas the duration of adolescent sterility did not differ between lesioned and control females. However, control females showed more variation in the age at menarche than did lesioned females. The increased variation in age at menarche in control females and the increased variation in age at first ovulation is likely why no difference in duration of adolescent sterility was found between lesioned and control females. In this study, several control females reached menarche early creating a distribution of age at menarche across two years, which is not consistent with previous research showing age at menarche is consistently around 2.5 years of age in socially-housed monkeys (Wilson et al., 1986; Zehr et al., 2005; Wilson et al., 2013). Previous research has begun sampling at 2.5 years of age (Wilson et al., 1986; Zehr et al., 2005), and thus, it is possible that menarche can and does occur earlier in some females but is not detected. In studies that began sampling in socially-housed rhesus females prior to 2.5 years of age, puberty onset was defined as menarche or first sexual swelling and thus, age at menarche between studies could not be compared (Wilson et al., 2013).

Neonatal bilateral amygdala lesions resulted in earlier puberty onset in female rhesus macaques and earlier first ovulation is likely a consequence of earlier menarche; however the mechanisms regulating earlier menarche in lesioned animals is unknown. One potential mechanism may involve the inhibition of GnRH release by *gamma*-Aminobutyric acid, GABA. GABA levels decline around the same time when the initial GnRH release occurs at puberty onset (Mitsushima, Hei, & Terasawa, 1994). In addition, chronic administration of bicuculine, a GABA receptor antagonist, to prepubertal juvenile female rhesus macaques resulted in earlier menarche and first ovulation (Keen, Burich, Mitsuhima, Kasuya, & Terasawa, 1999). Age at menarche in lesioned females in the current study was similar to the age at menarche in bicuculine-treated females (Keen et al., 1999). The amygdala contains GABAergic neurons (Pitkänen & Amaral, 1994) and thus, lesions of the amygdala may result in an earlier removal of GABA inhibition on GnRH release, which results in earlier menarche, and ultimately, earlier first ovulation. Future research is needed to determine how and if GABAergic neurons in the amygdala alter GnRH release and whether the decline in GABA prior to puberty is influenced by the amygdala.

Does the amygdala eliminate the effects of social rank on pubertal timing?

Social rank did not influence age at menarche or first ovulation in control females, which is inconsistent with previous data showing lower-ranking females experience later first ovulation (Zehr et al., 2005; Wilson & Kinkead, 2008; Wilson et al., 2013). Previous research has shown that the effects of delayed ovulation are limited to lower-ranking females, with high- and middle-ranking females showing greater variation in the age at first ovulation than low-ranking females. It is possible that we were not able to

detect a significant relationship between rank and first ovulation because we examined a linear relationship between social rank and age at first ovulation; however, the sample size in the current study was not large enough to group animals based on social rank as was done in previous research (Zehr et al., 2005). In addition, delayed first ovulation in lower-ranking females was found to be specific to females that were homozygous for a short promoter region on the serotonin reuptake transporter gene (Wilson & Kinkead, 2008). In the current study, none of the females, lesioned or controls, were homozygous for the short promoter region on this gene. Thus, it is also possible that we did not detect an effect of social rank on age at first ovulation in our control females because the gene-environment interaction that results in delayed ovulation was not present.

Due to the lack of significant relationship between social rank and pubertal timing in control females, it is difficult to determine whether lesions of the amygdala eliminated the influence of social cues on pubertal timing. If a relationship between social rank and pubertal timing existed in control females, but not in lesioned females, that would suggest that lesions of the amygdala eliminated the effects of social information on pubertal events. However, without this relationship between social rank and pubertal events in control females, we cannot say with any certainty whether or not the amygdala is important for modulating social information that influences pubertal timing.

Does the amygdala influence sexual behavior and how do these effects differ based on the social context?

Sexual behavior was examined in lesioned females in both pair tests and social group observations and the effects of amygdala damage on sexual behavior varied based on the social context. Pair tests typically result in a consistent level of mounting by the

male across the ovarian cycle (Goy, 1979). In the current study, female sexual receptivity during pair tests was not altered by amygdala lesions as there was no difference in rates of mounting by the male between lesioned and control females, which is consistent with data from adult-lesioned females (Spies et al., 1976). However, the rates of mounting observed during the pair tests were low, which may have precluded detecting an effect.

With respect to female-initiated behaviors during the pair tests, lesioned females showed either no difference in male-directed behaviors or a greater frequency/duration of female-initiated, male-directed behaviors in comparison to control females. For example, lesioned females consistently showed higher rates of approaching within one meter of the male and following the male in comparison to control females. Thus, lesioned females were not observed to have lower levels of female-initiated sexual behavior than control females during the pair tests, but rather for certain behaviors, showed higher levels of interest in the male. When estradiol levels were related to rates of behavior, the relationship only existed in control females, indicating that behavior was not modulated by estradiol levels in lesioned females.

Spies and colleagues (1976) found that female sexual behavior was not altered following amygdala lesion surgery, with the exception of a decrease in initiating proximity to the male. The results from the pair tests in the current study are consistent with the findings of Spies and colleagues, except for the decreased levels of female-initiated proximity observed. In contrast to the findings of Spies and colleagues, lesioned females in the current study did not show a decrease in initiating proximity to the male. One possible explanation for this difference in female-initiated proximity is

that females in the previous study received lesions in adulthood and were sexually-experienced prior to amygdala lesion surgery, whereas in the current study, lesions occurred at one month of age. It is possible that lesions of the amygdala during adulthood interfered with the relationship between estradiol levels and behavior, as was evident in the current study, and that decreased proximity initiation following amygdala lesions was a result of reduced sensitivity to estradiol in modulating the female's behavior. Future research is needed to determine whether lesions of the amygdala influence sexual behavior as a result of reduced sensitivity to estradiol.

In contrast to the pair tests, where neonatal treatment differences were the result of higher rates of sexual behavior in lesioned females, in social group observations, differences in behavior between lesioned and control females were the result of control females showing higher levels of behavior. Thus, the direction of behavioral differences between lesioned and control females differed based on the social context. As predicted, when differences in female-initiated behavior existed between lesioned and control females in the social group observations, lesioned females showed lower rates of behavior, such as lower rates of proximity to the male, in comparison to control females. Despite the lower rates of certain behaviors observed in lesioned females, mounting by the male did not differ between lesioned and control females, indicating that lesions of the amygdala did not interfere with female sexual receptivity, as was seen in the pair tests and in previous research (Spies et al., 1976). The lack of a difference in rates of mounting during the follicular phase is interesting considering that lesioned females showed lower rates of proximity to the adult male. It was predicted that less mating would occur in social groups in lesioned females because lesioned females would not be

driving the mating interactions. The lack of a difference in mounting by adult males may suggest that males are initiating the interactions with lesioned females. However, it seems more likely that control females are more motivated, as evidenced by higher rates of sexual solicits in control females, to attract the male and thus, spend more time in proximity with the male. Similar to the findings in the pair tests, in the social group observations, estradiol levels were only related to behaviors in the control females. Thus, lesioned females may show lower levels of female-initiated, male-directed behaviors in the social group because estradiol is not modulating behavior during the follicular phase in lesioned females.

In summary, lower rates of female-initiated behaviors in lesioned females were only apparent in social group observations. Thus, in female rhesus macaques, the amygdala is important for aspects of female sexual behavior when the social context requires that females compete with other females for mating opportunities. Estradiol did not modulate behavior in lesioned females, even when significant relationships were found in control females, which may in part be the mechanism by which behavior is influenced in lesioned females. Despite differences in female proceptivity, female sexual receptivity was not altered in lesioned females, regardless of social context. These data suggest that the amygdala is important for female-initiated behaviors, perhaps involving the motivation to attract a mate, but that the amygdala is not required for mating to occur.

Implications & Future Directions

The current study demonstrates that the amygdala is capable of influencing puberty onset and sexual behavior thus, it is possible that the amygdala modulates social, contextual information that influences reproduction. Earlier puberty onset in girls

following parental divorce, or the presence or absence of a father is thought to be largely driven by differences in body weight (Wierson et al., 1993; Graber et al., 1995). However, the current study suggests not only that greater body weight is not always predictive of earlier puberty, but also that the amygdala may be important for modulating these social effects on puberty onset in girls independent of body weight. Future research is needed to determine specifically if social context information that influences reproduction is modulated by the amygdala and the neurobiological mechanisms that are altered, such as GABA inhibition, as a result of the social context.

In men and women, activation of the amygdala occurs when viewing sexually explicit images, which suggests that the amygdala is involved in at least some aspects of human sexual behavior (Hamann et al., 2004). Data from the current study suggest that aspects of female sexual initiation are influenced by the amygdala, primarily in social contexts where the female must compete for the male. Thus, it is possible that one function of the amygdala is to provide salence to sexual cues, thereby motivating the female to mate with the male. Another possibility is that the amygdala is important for modulating the relationship between estradiol levels and sexual behavior. Future research is needed to determine the mechanism(s) by which the amygdala influences sexual behavior.

Conclusions

Neonatal amygdala lesions resulted in earlier puberty onset and first ovulation in female rhesus macaques living in large social groups, indicate that the amygdala is capable of influencing the timing of puberty onset. Although the exact mechanism for earlier puberty onset in lesioned females is unknown, it is possible that GABAergic

mechanisms regulating GnRH release are involved. Certain female-initiated sexual behaviors were lower in lesioned females, in comparison to control females, during social group observations and these effects were specific to the social context. Estradiol does not appear to modulate female sexual behavior in lesioned females and future research is needed to determine if lesioned females are less sensitive to the effects of estradiol.

General Introduction and Discussion References

Abbott, D. H., Hodges, J. K., & George, L. M. (1988). Social status controls LH secretion and ovulation in female marmoset monkeys (*Callithrix jacchus*). *Journal of Endocrinology*, *117*, 329-339.

Abbott, D. H., McNeilly, A. S., Lunn, S. F., Hulme, M. J., & Burden, F. J. (1981). Inhibition of ovarian function in subordinate female marmoset monkeys (*Callithrix jacchus jacchus*). *Journal of Reproduction and Fertility*, *63*, 335-345.

Adams, D., Gold, A. R., & Burt, A. D. (1978). Rise in female-initiated sexual activity at ovulation and its suppression by oral contraceptives. *New England Journal of Medicine*, *299*, 1145-1150.

Amaral, D. G., Price, J. L., Pitkanen, A., & Carmichael, S. T. (1992). Anatomical Organization of the Primate Amygdaloid Complex, In. J.P. Aggleton, (Ed.), *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction* (pp.1-66). New York: Wiley-Liss, Inc.

Barrett, J., Abbott, D. H., & George, L. M. (1990). Extension of reproductive suppression by phermonal cues in subordinate female marmoset monkeys, *Callithrix jacchus*. *Journal of Reproduction and Fertility*, *90*, 411-418.

Baxter, M. G., & Murray, E. A. (2002). The amygdala and reward. *Nature Reviews Neuroscience*, *3*, 563-573.

Beach, F. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. *Hormones and Behavior*, *7*, 105-138.

Beltramino, C., & Taleisnik, S. (1978). Facilitatory and inhibitory effects of electrochemical stimulation of the amygdala on the release of luteinizing

hormone. *Brain Research*, *144*, 95-107.

Bennett, A. L., Greco, B., Blasberg, M. E., & Blaustein, J. D. (2002). Response to male odours in progestin receptor- and oestrogen receptor-containing cells in female rat brain. *Journal of Neuroendocrinology*, *14*, 442-449.

Bercovitch, F. B., & Strum, S. C. (1993). Dominance rank, resource availability, and reproductive maturation in female savanna baboons. *Behavioral Ecology and Sociobiology*, *33*, 313-318.

Bermant, G., & Westbrook, W. H. (1966). Peripheral factors in the regulation of sexual contact by female rats. *Journal of Comparative Physiology & Psychology*, *61*, 244-250.

Bloch, G. J., & Ganong, W. F. (1971). Lesions of the brain and the onset of puberty in the female rat. *Endocrinology*, *89*, 898-901.

Bruce, H. M. (1959). An exteroceptive block to pregnancy in the mouse. *Nature*, *184*, 105.

Butcher, R. L., Collins, W. E., & Fugo, N. W. (1974). Plasma concentrations of LH, FSH, prolactin, progesterone, and estradiol-17B throughout the 4-day estrous cycle of the rat. *Endocrinology*, *94*, 1704-1708.

Carrillo, A. J., Rabii, J., Carrer, H. F., & Sawyer, C. H. (1977). Modulation of the proestrous surge of luteinizing hormone by electrochemical stimulation of the amygdala and hippocampus in the unanesthetized rat. *Brain Research*, *128*, 81-92.

Chateau, D., Kauffmann, M. T., & Aron, C. (1984). Are the amygdaloid projections to the hypothalamic ventromedial nucleus involved in estrous rhythm regulation in the female rat? *Experimental and Clinical Endocrinology*, *83*, 303-309.

Chateau, D., Roos, J., Plas-Roser, S., Roos, M., & Aron, C. (1976). Hormonal mechanisms involved in the control of oestrous cycle duration by the odour of urine in the rat. *Acta Endocrinologica, 82,* 426-435.

Chongthammakun, S., & Terasawa, E. (1993). Negative feedback effects of estrogen on luteinizing hormone-releasing hormone release occur in pubertal, but not prepubertal, ovariectomized female rhesus monkeys. *Endocrinology, 132,* 735-743.

Cowan, W. M., Raisman, G., & Powell, T. P. (1965). The connexions of the amygdala. *Journal of Neurology, Neurosurgery, and Psychiatry, 28,* 137-151.

Döcke, F. (1974). Differential effects of amygdaloid and hippocampal lesions on female puberty. *Neuroendocrinology, 14,* 345-350.

Döcke, F. (1981). The limbic system and female maturation. *Experimental Brain Research, Supplement 3,* 208-221.

Döcke, F., & Bao, T. N. (1978). Increased sensitivity to estrogen of the bed nucleus of the stria terminalis following deefferentation of the mediocortical amygdala: Evidence for a compensatory ovulatory mechanism. *Endokrinologie, 72*(3), 257-264.

Döcke, F., Lemke, M., & Okrasa, R. (1976). Studies on the puberty-controlling function of the mediocortical amygdala in the immature female rat. *Neuroendocrinology, 20,* 166-175.

Döcke, F., Rohde, W., & Dörner, G. (1983). Increase of FSH secretion in immature female rats following lesioning of the medial amygdaloid nucleus. *Experimental and Clinical Endocrinology, 82,* 384-387.

Döcke, F., Rohde, W., Lange, T., & Dörner, G. (1980). Evidence for direct central nervous inhibition of LH secretion during sexual maturation of female rats. *Endokrinologie*, *75*, 1-7.

Eisner, J. R., Dumesic, D. A., Kemnitz, J. W., & Abbott, D. H. (2000). Timing of prenatal androgen excess determines differential impairment in insulin secretion and action in adult female rhesus monkeys. Journal of Clinical Endocrinology & Metabolism, 85, 1206–1210.

Ellendorff, F., Colombo, J. A., Blake, C. A., Whitmoyer, D. I., & Sawyer, C. H. (1973). Effects of electrical stimulation of the amygdala on gonadotropin release and ovulation in the rat. *Proceedings of the Society for Experimental Biology and Medicine*, *142*(2), 417-420.

Erikson, L. B., & Wada, J. A. (1970). Effect of lesions in the temporal lobe and rhinencephalon on reproductive function in adult female rhesus monkeys. *Fertility & Sterility*, *21*, 434-454.

Foster, D. L. (1977). Luteinizing hormone and progesterone secretion during sexual maturation of the rhesus monkey: short luteal phases during initial menstrual cycles. *Biology of Reproduction*, *17*, 584-590.

Gallo, R. V., & Osland, R. B. (1976). Electrical stimulation of the arcuate nucleus in ovariectomized rats inhibits episodic luteinizing hormone (LH) release but excites LH release after estrogen priming. *Endocrinology*, *99*, 659-668.

Goodman, R. L. & Knobil, E. (1981). The sites of action of ovarian steroids in the regulation of LH secretion. *Neuroendocrinology*, *32*, 57-63.

Goy, R. W. (1979). Sexual compatibility in rhesus monkeys: predicting sexual behavior

of oppositely sexed pairs of adults. *Ciba Foundation Symposium, 62*, 227-255.

Guarraci, F. A., Megroz, A. B., & Clark, A. S. (2004). Paced mating behavior in the female rat following lesions of the three regions responsive to vaginocervical stimulation. *Brain Research, 999*, 40-52.

Hoffman, J. C. (1968). Effect of photoperiod on estrous cycle length in the rat. *Endocrinology, 83*, 1355-1357.

Hunt, P. A., & Hassold, T. J. (2008). Human female meiosis : What makes a good egg go bad ? Trends in Genetics, 24, 86-93.

Johnson, D. F., & Phoenix, C. H. Hormonal control of female sexual attractiveness, proceptivity, and receptivity in rhesus monkeys. *Journal of Comparative Physiology and Psychology, 90*, 473-483.

Kamberi, I. A., deVellis, J., Bacleon, E. S., & Inglish, D. (1980). Hormonal patterns of the hypothalamo-pituitary-gonadal axis in the rat during postnatal development and sexual maturation. *Endokrinologie, 75*, 129-140.

Kaplowitz, P. B., Slora, E. J., Wasserman, R. C., Pedlow, S. E., & Herman-Giddens, M. E. (2001). Earlier Onset of Puberty in Girls: Relation to Increased Body Mass Index and Race. *Pediatrics, 108*(2), 347-353.

Kawakami, M., & Higuchi, T. (1980). Comparison of the effect of electrochemical stimulation of the medial preoptic area and the hypothalamic arcuate nucleus upon LH release in ovariectomized and proestrous rats. *Endocrinologia japonica, 27*(2), 239-246.

Kawakami, M. & Kimura, F. (1975). Inhibition of ovulation in the rat by electrical stimulation of the lateral amygdala. *Endocrinologia japonica, 22*, 61-65.

Kawakami, M., Kimura, F., & Kawagoe, S. (1976). Cholinergic and serotonergic neural links and the inhibitory effects of hippocampus, lateral amygdala and central gray matter on gonadotropin release. *Endocrinologia japonica*, *23*, 11-21.

Kawakami, M., & Terasawa, E. (1972). Electrical stimulation of the brain on gonadotropin secretion in the female prepuberal rat. *Endocrinologia japonica*, *19*(4), 335-347.

Kawakami, M., Terasawa, E., Kimura, F., & Wakabayashi, K. (1973). Modulating effect of limbic structures on gonadotropin release. *Neuroendocrinology*, *12*, 1-16.

Keen, K. L., Burich, A. J., Mitsushima, D., Kasuya, E., & Terasawa, E. (1999). Effects of pulsatile infusion of the $GABA_A$ receptor blocker bicuculline on the onset of puberty in female rhesus monkeys. *Endocrinology, 140*, 5257-5266.

Kling, A., & Cornell, R. (1971). Amygdalectomy and social behavior in the caged stump-tailed macaque (*M. speciosa*). *Folia Primatologica*, *14*, 91-103.

Knobil, E. (1974). On the control of gonadotropin secretion in rhesus monkeys. *Recent Progress in Hormone Research*, *30*, 1-46.

Kondo, Y., & Sakuma, Y. (2005). The medial amygdala controls the coital access of female rats: A possible involvement of emotional responsiveness. *Japanese Journal of Physiology, 55*, 345-353.

Krey, L. C., Butler, R. C., & Knobil, E. (1975). Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. I. Gonadotropin secretion. *Endocrinology, 96*, 1073-1087.

Libertun, C., Orias, R., McCann, S. M. (1974). Biphasic effect of estrogen on the sensitivity of the pituitary to luteinizing hormone-releasing factor (LRF).

Endocrinology, 94, 1094-1100.

Mascó, D. H., & Carrer, H. F. (1980). Sexual receptivity in female rats after lesion or stimulation in different amygdaloid nuclei. *Physiology & Behavior, 24,* 1073-1080.

Mascó, D. H., & Carrer, H. F. (1984). Pathways conducting amygdaloid influence on feminine sexual behavior in the rat. *Behavioral Brain Research, 11,* 205-212.

Mathews, D., Donovan, K. M., Hollingsworth, E. M., Hutson, V. B., & Overstreet, C. T. (1983). Permanent deficits in lordosis behavior in female rats with lesions of the ventromedial nucleus of the hypothalamus. *Experimental Neurology,79,* 714-719.

Mitsushima, D., Hei, D. L., & Terasawa, E. (1994). γ-Aminobutyric acid is an inhibitory neurotransmitter restricting the release of luteinizing hormone-releasing hormone before the onset of puberty. *Proc Natl Acad Sci U S A, 91,* 395-399.

Moffit, T. E., Caspi, A., Belsky, J., & Silva, P. A. (1992). Childhood experience and the onset of menarche: A test of a sociobiological model. *Child Development, 63,* 47-58.

Mathews, D., & Edwards, D. A. (1977). Involvement of the ventromedial and anterior hypothalamic nuclei in the hormonal induction of receptivity in the female rat. *Physiology & Behavior, 19,* 319-326.

Nakai, Y., Plant, T. M., Hess, D. L., Keogh, E. J., & Knobil, E. (1978). On the sites of the negative and positive feedback actions of estradiol in the control of gonadotropin secretion in the rhesus monkey. *Endocrinology, 102,* 1008-1014.

Norman, R. L., & Spies, H. G. (1981). Brain lesions in infant female rhesus monkeys: Effects on menarche and first ovulation and on diurnal rhythms of prolactin and

cortisol. *Endocrinology*, *108*(5), 1723-1729.

Oomura, Y., Aou, S., Koyama, Y., Fujita, I., & Yoshimatsu, H. (1988). Central control of sexual behavior. *Brain Research Bulletin*, *20*, 863-870.

Paredes, R. G., & Vazquez, B. (1999). What do female rats like about sex? Paced mating. *Behavioural Brain Research*, *105*, 117-127.

Pasley, J. N., Powell, E. W., Cernosek, R. M. G., & Cernosek, S. F. (1978). Effects of amygdaloid lesions on reproductive function of group caged mice. *Neuroendocrinology*, *25*, 77-83.

Payne, C., Machado, C. J., Bliwise, N. G., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in *Macaca mulatta*: A volumetric magnetic resonance imaging study. *Hippocampus*, *20*, 922-935.

Petrulis, A., & Johnston, R. E. (1999). Lesions centered on the medial amygdala impair scent-marking and sex-odor recognition but spare discrimination of individual odors in female golden hamsters. *Behavioral Neuroscience*, *113*(2), 345-357.

Pfaff, D. (1970). Nature of sex hormone effects on rat sex behavior: Specificity of effects and individual patterns of response. *Journal of Comparative and Physiological Psychology*, *73*(3), 349-358.

Pfaus, J. G., Smith, W. J., & Coopersmith, C. B. (1999). Appetitive and consummatory sexual behaviors of female rats in bilevel chambers, *Hormones & Behavior*, *35*, 224-240.

Pitkänen, A. (2003). Connectivity of the rat amygdaloid complext, In. J.P. Aggleton, (Ed.), *The Amygdala: A functional analysis* (pp.31-115). New York: Oxford University Press.

Pitkänen, A., & Amaral, D. G. (1994). The distribution of GABAergic cells, fibers, and terminals in the monkey amygdaloid complex: an immunohistochemical and in situ hybridization study. *The Journal of Neuroscience, 14*(4), 2200-2224.

Plant, T. M. (2001). Neurobiological bases underlying the control of the onset of puberty in the rhesus monkey: A representative higher primate. *Frontiers in Neuroendocrinology, 22*, 107-139.

Plant, T. M., Krey, L. C., Moossy, J., McCormack, J. T., Hess, D. L., & Knobil, E. (1978). The arcuate nucleus and the control of gonadotropin and prolactin secretion in the female rhesus monkey (Macaca mulatta). *Endocrinology, 102*, 52-62.

Rajendren, G., & Moss, R. L. (1993). The role of the medial nucleus of the amygdala in the mating-induced enhancement of lordosis in female rats: The interaction with luteinizing hormone-releasing hormone neuronal system. *Brain Research, 617*, 81-86.

Raper, J., Wallen, K., Bachevalier, J., & Sanchez, M. (in preparation). Basal cortisol levels after nenonatal amygdala lesions in developing rhesus monkeys.

Relkin, R. (1971a). Relative efficacy of pinealectomy, hypothalamic and amygdaloid lesions in advancing puberty. *Endocrinology, 88*, 415-418.

Relkin, R. (1971b). Absence of alteration in pubertal onset in male rats following amygdaloid lesioning. *Endocrinology, 88*, 1272-1274.

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*, 354-361.

Rosvold, H. E., Mirsky, A. F., & Pribram, K. H. (1954). Influence of amygdalectomy on social behavior in monkeys. *Journal of Comparative Physiology & Psychology*, *47*, 173-178.

Sahu, A., & Ghosh, A. (1982). Effect of grouping and sex on the estrous regulation of a wild rat, Bandicota bengalensis. *Biology of Reproduction*, *27*, 1023-1025.

Sanchez, M. A., & Dominguez, R. (1995). Differential effects of unilateral lesions in the medial amygdala on spontaneous and induced ovulation. *Brain Research Bulletin*, *38*, 313-317.

Singh, K., & Greenwald, G. (1967). Effectsof continuous light on the reproductive cycle of the female rat: Induction of ovulation and pituitary gonadotropins during persistent estrus. *Journal of Endocrinology*, *38*, 389-394.

Smith, T. E., Faulkes, C. G., & Abbott, D. H. (1997). Combined olfactory contact with the parent colony and direct contact with nonbreeding animals does not maintain suppression of ovulation in female naked mole-rats. *Hormones and Behavior*, *31*, 277-288.

Spies, H. G., Norman, R. L., Clifton, D. K., Ochsner, A., J., Jensen, J. N., & Phoenix, C. H. (1976). Effects of bilateral amygdaloid lesions on gonadal and pituitary hormones in serum and on sexual behavior female rhesus monkeys. *Physiology & Behavior*, *17*, 985-992.

Spiteri, T., Musatov, S., Ogawa, S., Ribeiro, A., Pfaff, D. W., & Agmo, A. (2010). The role of the estrogen receptor α in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety, and aggression. *Behavioural Brain Research*, *210*(2), 211-20.

Takahashi, M., Ford, J. J., Yoshinaga, K., & Greep, R. O. (1977). Ovulation in light-estrous rats induced by darkness. *Endocrinologia japonica*, *24*, 89-96.

Tardif, S. D. (1984). Social influences on sexual maturation of female *Saguinus Oedipus Oedipus*. *American Journal of Primatology*, *6*, 199-209.

Terasawa, E., Kurian, J. R., Keen, K. L., Shiel, N. A., Colman, R. J., & Capuano, S. V. (2012). Body weight impact on puberty: Effects of high-calorie diet on puberty onset in female rhesus monkeys. *Endocrinology*, *153*, 1696-1705.

Thompson, C., Schwartzbaum, J. S., & Harlow, H. F. (1969). Development of social fear after amygdalectomy in infant rhesus monkeys. *Physiology & Behavior*, *4*, 249-254.

Tyler, J. L., & Gorski, R. A. (1980). Effects of corticomedial amygdala lesions or olfactory bulbectomy on LH responses to ovarian steroids in the female rat. *Biology of Reproduction*, *22*, 927-934.

Vandenbergh, J. G. (1974). Social determinants of the onset of puberty in rodents. *The Journal of Sex Research*, *10*(3), 181-193.

Van der Lee, S., & Boot, L. M. (1955). Spontaneous pseudopregnancy in mice. *Acta physiologica et pharmacologica Neerlandica*, *4*(3), 442-444.

Van der Lee, S., & Boot, L. M. (1956). Spontaneous pseudopregnancy in mice II. *Acta physiologica et pharmacologica Neerlandica*, *5*(2), 213-215.

Van Horn, R. N. (1980). Seasonal reproductive patterns in primates. *Progress in Reproductive Biology*, *5*, 181-221.

Velasco, M. E., & Taleisnik, S. (1969). Release of gonadotropins induced by amygdaloid stimulation in the rat. *Endocrinology*, *84*, 132-139.

Velasco, M. E., & Taleisnik, S. (1971). Effects of the interruption of amygdaloid and hippocampal afferents to the medial hypothalamus on gonadotrophin release. *Journal of Endocrinology*, *51*, 41-55.

Walker, M. L., Gordon, T. P., & Wilson, M. E. (1983). Menstrual cycle characteristics of seasonally breeding rhesus monkeys. *Biology of Reproduction*, *29*, 841-848.

Walker, M. L., Wilson, M. E., & Gordon, T. P. (1984). Endocrine control of the seasonal occurrence of ovulation in rhesus monkeys housed outdoors. *Endocrinology*, *114*, 1074-1081.

Wallen, K. (1990). Desire and ability: Hormones and the regulation of female sexual behavior. *Neuroscience & Biobehavioral Reviews*, *14*, 233-241.

Wallen, K. (1999). Risky business: Social context and hormonal modulation of primate sexual desire. , In. K. Wallen & J. Schneider, (Eds.), *Reproduction in Context* (pp.289-323). New York: Wiley-Liss, Inc.

Wallen, K. (2001). Sex and context: Hormones and primate sexual motivation. *Hormones & Behavior*, *40*, 339-357.

Wallen, K., Winston, L. A., Gaventa, S., Davis-DaSilva, M., & Collins, D. C. (1984). Periovulatory changes in female sexual behavior and patterns of ovarian steroid secretion in group-living rhesus monkeys. *Hormones and Behavior*, *18*, 431-450.

Whitten, W.K. (1956). Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *Journal of Endocrinology, 13*, 399–404.

Wilson, M. E., Bounar, S., Godfrey, J., Michopoulos, V., Higgins, M., & Sanchez, M. (2013). Social and emotional predictors of the tempo of puberty in female rhesus monkeys. *Psychoneuroendocrinology*, *38*, 67-83.

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 & Fertility, 70*, 625-633.

Wilson, M. E., Gordon, T. P., & Collins, D. C. (1982). Variation in ovarian steroids associated with the annual mating period in female rhesus (Macaca mulatta). *Biology of Reproduction, 27*, 530-539.

Wilson, M. E., Gordon, T. P., & Collins, D. C. (1986). Ontogeny of luteinizing hormone secretion and first ovulation in seasonal breeding rhesus monkeys. *Endocrinology, 118*, 293-301.

Wilson, M. E., & Kinkead, B. (2008). Gene-environment interactions, not neonatal growth hormone deficiency, time puberty in female rhesus monkeys. *Biol Reprod, 78*, 736-743.

Xia, L., Van Vugt, D., Alston, E. J., Luckhaus, J., & Ferin, M. (1992). A surge of gonadotropin-releasing hormone accompanies the estradiol-induced gonadotropin surge in the rhesus monkey. *Endocrinology, 131*, 2812-2820.

Yahr, P., & Greene, S. B. (1992). Effects of unilateral hypothalamic manipulations on the sexual behaviors of rats. *Behavioral Neuroscience, 106*, 698-709.

Zehr, J. L., Maestripieri, D., & Wallen, K. (1998). Estradiol increases female sexual initiation independent of male responsiveness in rhesus monkeys. *Hormones and Behavior, 33*, 95-103.

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*,

1087-1094.

Ziegler, T. E., Savage, A., Scheffler, G., & Snowdon, C. T. (1987). The endocrinology of puberty and reproductive functioning in female cotton-top tamarins (*Saguinus oedipus*) under varying social conditions. *Biology of Reproduction*, *37*, 618-627.