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Neonatal amygdala lesions advance pubertal timing in female rhesus macaques

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Summary

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 large, species-typical, social groups received bilateral neurotoxic amygdala lesions at one month of age and pubertal timing was examined beginning at 14 months of age. Pubertal timing was affected in neonatal amygdala-lesioned females (Neo-A), such that they experienced significantly earlier menarche and first ovulation than did control females (Neo-C). Duration between menarche and first ovulation did not differ between Neo-A and Neo-C females, indicating earlier first ovulation in Neo-A females was likely a consequence of earlier menarche. Social rank of Neo-A females was related to age at menarche, but not first ovulation, and social rank was not related to either event in Neo-C females. It is more likely that amygdectomy affects pubertal timing through its modulation of GABA-ergic mechanisms rather than as a result of the removal of a social-contextual inhibition on pubertal timing.

Keywords

Rhesus monkey; Amygdala; Puberty; Menarche; Social context

Menarche, first menstruation, marks the onset of puberty in girls (Marshall and Tanner, 1969), and indicates the transition to adult function of the hypothalamic–pituitary–gonadal (HPG) axis (Grumbach and Styne, 2003). Human menarche has been occurring earlier over the last century for reasons that are unclear, but likely reflect alterations in the nutritional and/or social environment (Herman-Giddens, 2006; Morris et al., 2011). Individual timing of menarche is influenced by the environmental and social context, such that parental

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Conflict of interest

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divorce or an unfamiliar male living in the household accelerates menarche (Moffitt et al., 1992; Wierson et al., 1993; Graber et al., 1995; Ersoy et al., 2005), as well as by epigenetic factors, including the timing of mother's menarche (Graber et al., 1995; Ersoy et al., 2005). Thus, some system(s) monitor(s) the developing girl's physical and social environment and interacts with the HPG axis to regulate the timing of menarche and puberty onset. Yet, the brain mechanisms underlying this contextual variation in puberty are unknown.

Female rhesus macaques (*Macaca mulatta*), like humans, are menstrual primates with a faster developmental trajectory, but similar reproductive milestones to those seen in girls (Marshall and Tanner, 1969; Foster, 1977; Resko et al., 1982). Menarche in rhesus macaques, as in girls, marks the onset of a cascade of neuroendocrine events leading to adult reproductive competency (Foster, 1977; Resko et al., 1982). This pubertal cascade is affected by social context as higher-ranking females have an earlier menarche or first genital swelling than do lower-ranking females (Wilson et al., 2013); however, this relationship is not consistently found (Zehr et al., 2005; Wilson and Kinkade, 2008). By contrast, lower-ranking females consistently experience later first ovulation than do high- or middle-ranking females (Zehr et al., 2005; Wilson et al., 2013). In addition to altering pubertal timing, social rank may also influence variation in pubertal timing as low-ranking females showed less variation in age at first ovulation than did high- or middle-ranking females (Zehr et al., 2005). Thus, social context modulates the activation of HPG axis function, but as in humans, the neural modulatory mechanism(s) are unknown.

The amygdaloid complex is a candidate for integrating social contextual information with HPG axis function (Rosvold et al., 1954; Thompson et al., 1969; Kling and Cornell, 1971; Amaral et al., 1992; Petrulis and Johnston, 1999). In female rats, amygdala lesions alter the timing of pubertal onset with the direction of the effect varying with the developmental timing of the lesion. Bilateral lesions of the anterior medial amygdala in 15-day-old female rats delay pubertal onset, lesions of 21-day-old rats result in earlier pubertal onset, but lesions at 26 days of age do not affect pubertal onset (Döcke, 1974; Döcke et al., 1976, 1980). In individually-housed female rhesus monkeys, lesions of the entire amygdala at 10–13 months of age, after the amygdala is fully developed (Payne et al., 2010), do not influence menarchal age (Norman and Spies, 1981). Little is known about how amygdala development in primates influences the timing of puberty onset.

The current study focuses on the effects of neonatal neurotoxic amygdala lesions on the timing of menarche and first ovulation in rhesus macaque females reared in large, species-typical social groups. If social context produces variable pubertal timing, which it appears to, and if the amygdala mediates the effects of social context on pubertal timing, then neonatally amygdalectomized females should have less variability in age at menarche and first ovulation than females with intact amygdala function. Thus, we predicted that neonatal amygdalectomy would likely lead to earlier pubertal timing and reduce the variation in age at menarche and first ovulation in comparison to control females.

1. Method

1.1. Subjects

Subjects were female rhesus macaques ($N = 16$; born March–June) living with their mothers and siblings in large, species-typical social groups at the Yerkes National Primate Research Center (YNPRC) Field Station (Lawrenceville, GA). Subjects were selected from high-, middle-, and low-ranking matriline, excluding the highest- and lowest-ranking matriline, so that females had comparable social contexts, with all females having matriline ranked above and below their matriline. Social groups consisted of 75–100 animals, including approximately 25 adult females, their offspring under three years of age, and two adult males. Subjects were housed in 38 m $\times$ 38 m 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 ($M = 27.14 \pm 0.74$ days of age), whereas Neo-C females ($M = 24.17 \pm 1.99$ days of age) received a sham surgery, consisting of inhalation anesthesia and surgical opening and suturing of the scalp. Neo-BC females ($M = 28.33 \pm 1.20$ days of age) received 24 h separation from their mothers duplicating the separation of the other two groups of females, and a 2 h period of ketamine anesthesia, without any scalp surgery. Following surgery or behavioral sham manipulations, subjects returned to their social group where they remained housed except during removal for short experimental procedures or for medical care. Due to colony management changes in social groups, one Neo-A female was temporarily housed individually for a two-month period during data collection, but neither menarche nor first ovulation occurred during this time period. Two Neo-A and two Neo-C females were housed in a mixed-sex peer group during the breeding season at 1.5 years of age. All procedures were approved by the Institutional Animal Care and Use Committee at Emory University and followed the Guide for the Care and Use of Laboratory Animals by the National Institute of Health.

1.2. Surgical procedures

1.2.1. Neonatal amygdala lesion (Neo-A) surgery and procedure—The amygdala lesion surgery procedure has been previously described (Raper et al., 2013a). Briefly, subjects and their mothers were removed from the social group and transported to the YNPRC Main Station. On the morning of surgery, the infant was separated from the mother, anesthetized (Ketamine hydrochloride, 1 mg/kg BW, i.m.), intubated, and given isoflurane (1–2% to effect) throughout the neuroimaging and surgical procedures. 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] = 11 ms, repetition time [TR] = 450 ms, contiguous 4 mm sections, 12 cm field of view [FOV], 256 $\times$ 256 matrix) were taken at 1 mm slices throughout the brain. In addition, three fluid attenuated inversion recovery (FLAIR) scans (3D T1-weighted fast spoiled gradient [FSPGR]-echo sequence, TE = 2.6 ms, TR = 10.2 ms, 25 flip angle, 12 cm FOV, 256 $\times$ 256 matrix) were acquired at 3

mm, with each scan staggered 1 mm posterior, yielding 1 mm coronal sections throughout the brain.

Four injection sites (1 mm 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 (1 mm lateral and medial or 1 mm lateral, medial, and dorsal to the center of the amygdala) were added using T1 images immediately 1 mm anterior and 1 mm 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 7.4, 10 mg/ml concentration) injections (6–10 injections per hemisphere; 0.6–0.8 μ l/injection) occurred simultaneously in each hemisphere at a rate of 0.2 μ l/min. To prevent the ibotenic acid from spreading beyond the amygdala, the needle remained in place for 3 min 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. Throughout the neuroimaging and surgical procedures, body temperature was maintained via a heating pad and all vital signs were continuously recorded. Following surgery, all animals received medications to prevent pain (banamine; 1 mg/kg for 3 days), edema (dexamethosone; 0.5 mg/kg for 3 days), and infection (rocephin; 25 mg/kg for 7 days). T1 and FLAIR coronal images were collected one week after surgery under anesthesia as previously described (Section 1.2.1) and were compared to images prior to surgery to determine the location and extent of the lesion (Malkova et al., 2001; Nemanic et al., 2002).

1.2.2. Sham-operated (Neo-C) surgery and procedure—The same treatments and procedures were performed on Neo-C females as were performed on Neo-A females, with the exception of two procedures. Neo-C animals did not have a needle lowered into the amygdala in order to avoid possible brain hemorrhages and thus, did not receive any injections into the amygdala. Secondly, Neo-C animals did not receive post-surgical MRI scans and thus, Neo-C females were not anesthetized one week following surgery. Several laboratories have used this procedure as a surgical control in primate research (Bauman et al., 2004; Kazama and Bachevalier, 2012). 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 that Neo-A animals were separated from their mothers.

1.2.3. 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 YNPRC Field Station where the mother-infant pair was housed for the remainder of the procedure. The following day, the infant was separated from its mother, anesthetized

(Ketamine hydrochloride, 1 mg/kg BW, i.m.), and had its head shaved and cleaned with Nolvasan solution as was the case for infants undergoing surgery. Neither MRI scans nor surgical procedures were performed on Neo-BC animals. Upon recovery from anesthesia, the infant received the same treatment as Neo-C animals, which included post-operative medications, 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.

1.3. Mother–infant reunions

After recovery from anesthesia, the infant was housed individually in the nursery overnight in an incubator ventilated with oxygen. The next morning, the infant was returned to its mother and continuously monitored via a web camera to determine whether the infant was nursing. Overnight separations with the infant returning to the nursery and morning reunions with the mother occurred until the animal was observed comfortably nursing to ensure the animal's health (Neo-A: 4 ± 0.5 reunions (excluding one female later discussed in this section); Neo-C: 1.7 ± 0.5 reunions; Neo-BC: 1.0 ± 0 reunions). Infants were supplemented with formula during overnight separations from the mother.

After the post-surgical MRI scans (Neo-A females) or separation from mother one week following the initial procedure (Neo-C and Neo-BC females) and when the infant was nursing regularly, the pair was returned to their social group at the YNPRC Field Station, where the pair was closely monitored to ensure both the mother and infant successfully reintegrated into the group. The total time spent out of the social group significantly differed by neonatal treatment (Neo-A: 21 ± 1.7 days; Neo-C: 19 ± 1.6 days; Neo-BC: 13 ± 0.7 days; $F(2,13) = 4.84$, $p = .027$), but follow-up Bonferroni comparisons revealed only a significant difference in time spent out of the social group between Neo-A and Neo-BC females, $p = .025$, with no significant difference found between Neo-C and Neo-BC females, $p = .153$.

In one case (Neo-A female), the mother did not accept the infant after surgery despite repeated reunion attempts. Upon release with its mother into the social group, this Neo-A infant was retrieved and adopted by another adult female who had already had an infant of a similar age (not part of the current study). The adult female and “twins” were closely monitored to ensure that both infants were nursing and adequately gaining weight. The time this female spent out of the social group was within the range of the other Neo-A females.

1.4. Amygdala 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., 2013a). Using the program Image-J, the surface area of the damage was calculated for each image and multiplied by 1 mm, the thickness of each section, to calculate the volume of damage. The volume of damage was divided by the total volume of the amygdala, calculated using a template brain, to determine the percentage of damage to the amygdala. For each case, extent of damage to the amygdala (Table 1) was calculated for the right hemisphere, left hemisphere, average damage (average of right and left damage), and damage shared by both hemispheres (right damage $\times$ left damage)/

100). An example case of the bilateral amygdala lesion extent, using FLAIR images, is presented in Fig. 1. Extent of inadvertent damage to the hippocampus and area TE were less than 4% and 1%, respectively (Raper et al., 2013a).

1.5. Data collection procedure

All subjects were trained as juveniles to separate from the group and move to an indoor catch area on command (Raper et al., 2013b). Once in the catch area, subjects were transferred to a temporary cage, which contained holes allowing 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 et al., 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 and 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 (subjects were born from March to June). 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 11:30 h and 15:30 h to detect luteal increases in progesterone.

1.6. Age at menarche and 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 when the subject was 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 2 ng/ml for a minimum of seven days or above 5 ng/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).

1.7. Social rank

Social rank for juvenile females reflects their mother's social rank and thus, maternal rank in August was a proxy for juvenile rank, at the start of data collection, when subjects were 14–17 months of age (juvenile social rank). Social rank was assigned by dividing the rank of the mother by the total number of adult females in the group, compensating for different group sizes. Two Neo-A and two Neo-C females (data indicated in Fig. 2) were housed in a mixed-sex peer group during the breeding season at 1.5 years of age (as described in Section 2.1) and juvenile social rank was calculated by dividing the female's social rank by the total number of animals in the group.

1.8. Body weight

Greater juvenile body weight has been related to earlier pubertal measures in female rhesus macaques (Terasawa et al., 2012). Thus, differences in pubertal measures between amygdala-lesioned and control females may result from differences in juvenile body weight. To examine the effect that juvenile body weight may have on pubertal timing, mean monthly body weights from 15 to 21 months of age were used to examine differences between neonatal treatment groups. The mean body weight from 15 to 21 months of age (mean juvenile body weight), was used in regression and correlation analyses. Body weights at menarche and first ovulation were calculated by averaging weekly body weights for the four week period prior to the pubertal event.

1.9. Hormonal assays

Blood samples were collected in EDTA tubes (BD Vacutainer, #366431), centrifuged (Beckman-Coulter, Allegra 6R) at 3000 rpm for 15 min at 4°C, and the plasma was stored at -20°C until assayed. Radioimmunoassay commercially prepared kits (Siemens Healthcare, Los Angeles, CA) were used to determine progesterone levels, with a lower sensitivity limit of 0.10 ng/ml and inter- and intra-assay coefficients of 13.6% and 8.4%, respectively. Hormone assays were completed by the Biomarkers Core Laboratory at the Yerkes National Primate Research Center.

1.10. Statistical analysis

Independent t-tests were used to examine differences in age at menarche and first ovulation between Neo-C and Neo-BC females. The proportion of control and amygdala-lesioned females experiencing menarche and first ovulation each season was compared using a chi-square test. Hierarchical linear model (HLM) regression analyses were completed separately for age (days) at menarche and first ovulation and duration of adolescent sterility (days) to determine if neonatal treatment (Neo-A, Neo-C), social rank, and/or body weight significantly predicted pubertal timing. Pearson's correlations were performed separately for Neo-A and Neo-C females to examine how social rank and body weight related to pubertal measures (menarche, first ovulation, duration of adolescent sterility). To determine if the variation in age at first ovulation differed by neonatal treatment, coefficients of variation (CV) (standard deviation/mean) were compared between neonatal treatment groups using the ratio of squared CVs as an *F* value (Sokal and Braumann, 1980; Wallen and Lloyd, 2008). Quantitative data are presented as means $\pm$ standard errors of the mean (SEM). A *p* < .05 is considered significant. Effect sizes estimates reported are ϕ for Chi-square statistics.

First ovulation data and thus, adolescent sterility duration, were not available for one Neo-A female (Neo-A-F6), who was one of the two Neo-A females housed in a mixed-sex peer group at 1.5 years of age. One Neo-A female (Neo-A-F2) was an outlier (> 2SD from the mean) and was excluded from analyses examining the relationships between mean juvenile body weight and pubertal measures in Neo-A females (data identified in Fig. 3).

Menstruation was intermittently assessed between the first and second breeding season (March to August). Thus, 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 females that did not reach menarche until the second breeding season, we analyzed the data first assuming that they reached menarche the first day after consistent sampling stopped and this age was used to compare age of menarche in amygdala-lesioned and control females. We then compared age at menarche using the age at the first detected menstrual bleeding in the following (second) 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 amygdala-lesioned females experienced menarche prior to the period of intermittent menstrual sampling.

2. Results

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

2.1. Menarche

All neonatal amygdala-lesioned females experienced early menarche, during the breeding season at 1.5 years of age, in comparison to only four of nine control females, $X^2(1, N = 16) = 5.66, p = .017, \phi = .59$ (Fig. 2a). The remaining control females experienced menarche on-time during the next breeding season, when 2.5 years of age. Age at menarche was significantly earlier in Neo-A than in Neo-C females, whether age at menarche was calculated conservatively, controlling for possibly missing menarche during intermittent sampling (see methods, 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 did not alter the results of the linear regression when neonatal treatment (Neo-A or Neo-C) was used to predict age at menarche (conservative age at menarche: $F(1,15) = 6.59, p = .022$; actual age at menarche: $F(1,15) = 7.88, p = .014$). The method of calculating age at menarche did not alter finding significant differences between amygdala-lesioned and control females and therefore, the actual age at menarche was used in all subsequent analyses.

Neonatal treatment significantly predicted age at menarche, accounting for 36% of the variance in age at menarche, in that Neo-A females experienced menarche earlier than did Neo-C females (Table 2). Age at menarche within Neo-A females was significantly related to the amount of damage to the right amygdala (Table 1), with earlier menarche in Neo-A females with more right amygdala damage. Age at menarche was not significantly related to left, average, or shared amygdala damage (Table 1).

2.2. First ovulation

The proportion of females reaching first ovulation each breeding season did not differ between amygdala-lesioned and control females, $X^2(2, N = 15) = 4.39, p = .112, \phi = .54$ (Fig. 2a). Neo-A females ($CV = 39.00$) had a greater variation in age at first ovulation

in comparison to Neo-C females ($CV = 16.69$), $F(1,13) = 5.46$, $p = .036$. Amygdala-lesioned females' ovulated significantly earlier than did controls with neonatal treatment accounting for 30% of the variance in age at first ovulation (Table 2). Age at first ovulation was significantly related to left amygdala damage in that females with more left amygdala damage experienced later first ovulation (Table 1), but was not related to right, average, or shared amygdala damage (Table 1).

2.3. Adolescent sterility

Neonatal treatment did not significantly predict the duration of adolescent sterility (Table 2; Fig. 2b), despite neonatal treatment being a significant predictor for both age at menarche and first ovulation. Earlier age at first ovulation was related to a shorter period of adolescent sterility in both Neo-C females, $r(9) = .71$, $p = .033$, and Neo-A females, $r(6) = .99$, $p < .001$. Age at menarche and length of adolescent sterility were related in Neo-C females, $r(9) = -.67$, $p = .047$, 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, $r(9) = .044$, $p = .911$, suggesting that the duration of adolescent sterility is independently related to ages at menarche and first ovulation. The relationship between adolescent sterility length and age at menarche was not found in amygdala-lesioned females, $r(6) = -.05$, $p = .928$, possibly because all Neo-A females reached menarche in the same breeding season.

2.4. Social rank

For all females, juvenile social rank did not significantly predict age at menarche (Table 2), indicating that earlier menarche in Neo-A females is not the result of higher social rank in Neo-A females in comparison to Neo-C females. Because the effects of social rank on the timing of pubertal measures were expected to be absent in Neo-A females, the effects of social rank on menarche, first ovulation, and adolescent sterility were examined separately for Neo-A and Neo-C females. Juvenile social rank was significantly related to age at menarche in Neo-A females in that higher-ranking Neo-A females reached menarche earlier than did lower-ranking Neo-A females. However, right lesion extent, which significantly predicted age at menarche, was also related to juvenile social rank, $r(7) = -.91$, $p = .004$. It is unlikely that lesion surgery influenced juvenile social rank as right lesion extent was also related to social rank at birth, $r(7) = -.83$, $p = .02$. Thus, the independent effects of right lesion extent and juvenile social rank on age at menarche in neonatal amygdala-lesioned females are not clear and it is possible that these significant findings simply reflect small sample sizes. There was no significant relationship between age at menarche and social rank in Neo-C females (Table 2).

Similar to age at menarche, age at first ovulation and adolescent sterility duration were not predicted by juvenile social rank for all females, indicating social rank cannot explain earlier first ovulation in Neo-A females (Table 2). Neither age at first ovulation nor duration of adolescent sterility were related to juvenile social rank for either amygdala-lesioned or control females (Table 2).

2.5. Body weight

In all females, mean body weight at menarche was positively related to age at menarche, $r(16) = .92, p < .001$, in that females reaching menarche earlier weighed less at 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 age at menarche between Neo-A and Neo-C females. In addition to the 36% of variance explained by neonatal treatment, mean juvenile body weight accounted for an additional 17% of the variance, with both neonatal treatment and mean juvenile body weight accounting for a total of 53% of the variance in age at menarche (Table 2). However, contrary to expectations, a lower mean juvenile body weight, rather than a greater body weight, predicted earlier menarche. This relationship between juvenile body weight and age at menarche was not found within Neo-A or Neo-C females when Neo-A and Neo-C females were analyzed as separate groups (Table 2; Fig. 3a).

As with menarche, body weight at first ovulation was positively related to age at first ovulation when all females are considered together, $r(15) = .75, p = .001$, indicating a critical body weight was not required for first ovulation. Mean juvenile body weight did not significantly account for any additional variance in age at first ovulation to that accounted for by neonatal treatment (Table 2). In Neo-C females, there was no significant relationship between mean juvenile body weight and age at first ovulation (Table 2; Fig. 3b). By contrast, in Neo-A females, greater juvenile body weight was related to a later age at first ovulation (Table 2; Fig. 3b). There was no relationship between left amygdala damage and mean juvenile body weight when the outlier was included in the analysis, $r(6) = .06, p = .916$, or when excluded from the analysis, $r(5) = .84, p = .09$, though the correlation was much greater when the outlier was excluded.

The direction of the relationship between juvenile body weight and length of adolescent sterility differed between Neo-A and Neo-C females. Greater juvenile body weight was related to a shorter duration of adolescent sterility in Neo-C females, whereas greater juvenile body weight was related to a longer duration of adolescent sterility in Neo-A females (Table 2; Fig. 3c).

3. Discussion

Age at menarche typically occurs at 2.5 years of age in rhesus macaques (Resko et al., 1982; Wilson et al., 1988). Neonatal amygdalectomy resulted in menarche in all Neo-A females 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 amygdala-lesioned and control females, possibly because, opposite to our prediction, the variation in age at first ovulation was greater in amygdala-lesioned than in control females. However, neonatal treatment significantly predicted 30% of the variance in age at first ovulation, with amygdala-lesioned females experiencing an earlier age at first ovulation. These results conflict with previous data showing that juvenile bilateral amygdala lesions have no effect on age at menarche or first ovulation (Norman and Spies, 1981). These divergent results may reflect that Norman and Spies' study created lesions at 10–13 months of age, after amygdala development is almost complete (Payne et al., 2010; Chareyron et al., 2012),

compared to approximately one month of age in the current study, when the amygdala has the greatest volume increase and shows substantial morphological changes (Payne et al., 2010; Chareyron et al., 2012). These different effects of amygdala lesions on pubertal timing based on lesion timing are consistent with data in female rats showing that 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; Döcke et al., 1976, 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 and Spies' subjects were housed indoors on a constant light/dark cycle at puberty and all females experienced first ovulation in the same year, at 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 breeding 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, and/or an effect of living in a social group remains to be further investigated.

It is unlikely that the differences in social-housing influenced earlier menarche in Neo-A females as all Neo-A females reached menarche at 1.5 years of age regardless of social housing condition. Peer-housing might advance menarche as the two Neo-C females that were peer-housed reached menarche at 1.5 years of age, as did the Neo-A females, and differences in social-housing may affect the variation in the timing of pubertal measures in this study. It does not appear, however, that differences in social-housing can account for the uniformly earlier menarche in Neo-A females.

Previous work in socially-housed animals has found no relationship between social rank and age at menarche (Wilson and Kinkad, 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 Neo-A females, but this relationship is confounded by the finding that extent of right amygdala damage is related to both social rank and age at menarche. It is not likely that lesion surgery influenced juvenile social rank because maternal social rank at birth was significantly related to juvenile social rank as well as right lesion extent. Therefore, it was not possible to identify the independent effects that right amygdala damage and/or juvenile social rank have on age at menarche. Because it is possible that the relationships of right amygdala damage and/or juvenile social rank to age at menarche reflect small sample sizes, it is premature to reach any conclusions about how/if social rank or right amygdala damage influence age at menarche.

Low social rank has been previously found to delay 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 few 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 amygdala-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 Neo-C and

Neo-A females makes it difficult to examine whether the amygdala modulates social information and alters pubertal timing.

One proposed hypothesis to explain the variation in timing of puberty onset in girls is that a critical body weight must be reached before menarche occurs (Frisch and Reville, 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 menarche in girls and rhesus monkeys (Moffit et al., 1992; Terasawa et al., 2012), but this effect cannot explain earlier menarche in amygdala-lesioned females as smaller juvenile body weight predicted their earlier menarche. There was no relationship between juvenile body weight 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 of age did not differ between female rhesus monkeys that ovulated at 2.5 or 3.5 years of age (Wilson et al., 1986). Data in rhesus monkeys have either shown a positive (Zehr et al., 2005) or no relationship (Wilson et al., 1986) between body weight and age at first ovulation, and pubertal data in humans have focused on the relationship between body weight and menarche, not body weight and first ovulation (Moffit et al., 1992; Graber et al., 1995). Thus, it was unexpected that in Neo-A females, greater juvenile body weight 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 significantly related to juvenile body weight, which suggests that left lesion damage and juvenile body weight may have independent effects on age at first ovulation in Neo-A females.

Lesion status significantly predicted ages at menarche and first ovulation, with neonatal amygdala-lesioned females experiencing earlier timing of pubertal events. Adolescent sterility length however, did not differ between Neo-A and Neo-C females, suggesting that earlier pubertal onset did not alter the relative timing of pubertal events after menarche, but rather advanced the timing of the pubertal period in amygdala-lesioned females. However, age at menarche was not related to age at first ovulation in amygdala-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 amygdala-lesioned and control females.

Neonatal amygdala lesions result in earlier puberty onset, 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 et al., 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 et al., 1999). The amygdala contains GABA-ergic neurons that project to the hypothalamus (Pitkänen and 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 to evaluate

this hypothesis and to fully understand the mechanism by which the amygdala is able to influence the timing of menarche.

4. Conclusion

The current study demonstrates the amygdala can influence the HPG axis and that damage to the amygdala early in life can result in earlier pubertal onset and reproductive maturity.

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References

- Amaral, DG.; Price, JL.; Pitkanen, A.; Carmichael, ST. Anatomical organization of the primate amygdaloid complex. In: Aggleton, JP., editor. *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*. New York: Wiley-Liss Inc; 1992. p. 1-66.
- Bauman MD, Lavenex P, Mason WA, Capitanio JP, Amaral DG. The development of mother-infant interactions after neonatal amygdala lesions in rhesus monkeys. *J. Neurosci.* 2004; 24:711–721. [PubMed: 14736857]
- Blank MS, Gordon TP, Wilson ME. Effects of capture and venipuncture on serum levels of prolactin, growth-hormone and cortisol in outdoor compound-housed female rhesus monkeys (*Macaca mulatta*). *Acta Endocrinol.* 1983; 102:190–195. [PubMed: 6829259]
- Chareyron LJ, Lavenex PB, Lavenex P. Postnatal development of the amygdala: a stereological study in rats. *J. Comp. Neurol.* 2012; 520:3745–3763. [PubMed: 22523001]
- Döcke F. Differential effects of amygdaloid and hippocampal lesions on female puberty. *Neuroendocrinology.* 1974; 14:345–350. [PubMed: 4472060]
- Döcke F, Lemke M, Okrasa R. Studies on the puberty-controlling function of the mediocortical amygdala in the immature female rat. *Neuroendocrinology.* 1976; 20:166–175. [PubMed: 986578]
- Döcke F, Rohde W, Lange T, Dörner G. Evidence for direct central nervous inhibition of LH secretion during sexual maturation of female rats. *Endokrinologie.* 1980; 75:1–7. [PubMed: 7371601]
- Ersoy B, Balkan C, Gunay T, Egemen A. The factors affecting the relation between menarcheal age of mother and daughter. *Child Care Health Dev.* 2005; 31:303–308. [PubMed: 15840150]
- Foster DL. Luteinizing hormone and progesterone secretion during sexual maturation of the rhesus monkey: short luteal phases during initial menstrual cycles. *Biol. Reprod.* 1977; 17:584–590. [PubMed: 411527]
- Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. *Science.* 1970; 169:397–399. [PubMed: 5450378]
- Graber JA, Brooks-Gunn J, Warren MP. The antecedents of menarcheal age: heredity, family environment, and stressful life events. *Child Dev.* 1995; 66:346–359. [PubMed: 7750370]

- Grumbach, MM.; Styne, DM. Puberty: ontogeny, neuroendocrinology physiology and disorders. In: Larsen, PR.; Kronenberg, HM.; Melmed, SM.; Polonsky, KS., editors. Williams Textbook of Endocrinology. 10th ed.. Philadelphia: Saunders; 2003. p. 1115-1286.
- Herman-Giddens ME. Recent data on pubertal milestones in United States children: the secular trend toward earlier development. *Int. J. Androl.* 2006; 29:241–246. [PubMed: 16466545]
- Kazama AM, Bachevalier J. Preserved stimulus-reward and reversal learning after selective neonatal orbital frontal areas 11/13 or amygdala lesions in monkeys. *Dev. Cogn. Neurosci.* 2012; 2:363–380. [PubMed: 22494813]
- Keen KL, Burich AJ, Mitsushima D, Kasuya E, Terasawa E. Effects of pulsatile infusion of the GABA_A receptor blocker bicuculline on the onset of puberty in female rhesus monkeys. *Endocrinology.* 1999; 140:5257–5266. [PubMed: 10537156]
- Kling A, Cornell R. Amygdalectomy and social behavior in the caged stump-tailed macaque (*M. speciosa*). *Folia Primatol.* 1971; 14:91–103.
- Malkova L, Lex CK, Mishkin M, Saunders RC. MRI-based evaluation of locus and extent of neurotoxic lesions in monkeys. *Hippocampus.* 2001; 11:361–370. [PubMed: 11530840]
- Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch. Dis. Child.* 1969; 44:291–303. [PubMed: 5785179]
- Mitsushima D, Hei DL, Terasawa E. 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.* 1994; 91:395–399. [PubMed: 8278400]
- Moffit TE, Caspi A, Belsky J, Silva PA. Childhood experience and the onset of menarche: a test of a sociobiological model. *Child Dev.* 1992; 63:47–58. [PubMed: 1551329]
- Morris DH, Jones ME, Schoemaker MJ, Ashworth A, Swerdlow AJ. Secular trends in age at menarche in women in the UK born 1908–93: results from the breakthrough generation study. *Paediatr. Perinat. Epidemiol.* 2011; 25:394–400. [PubMed: 21649682]
- Nemanic S, Alvarado MC, Price RE, Jackson EF, Bachevalier J. Assessment of locus and extent of neurotoxic lesions in monkeys using neuroimaging techniques: a replication. *J. Neurosci. Methods.* 2002; 121:199–209. [PubMed: 12468009]
- Norman RL, Spies HG. Brain lesions in infant female rhesus monkeys: effects on menarche and first ovulation and on diurnal rhythms of prolactin and cortisol. *Endocrinology.* 1981; 108:1723–1729. [PubMed: 6783391]
- Payne C, Machado CJ, Bliwise NG, Bachevalier J. Maturation of the hippocampal formation and amygdala in *Macaca mulatta*: a volumetric magnetic resonance imaging study. *Hippocampus.* 2010; 20:922–935. [PubMed: 19739247]
- Petrulis A, Johnston RE. Lesions centered on the medial amygdala impair scent-marking and sex-odor recognition but spare discrimination of individual odors in female golden hamsters. *Behav. Neurosci.* 1999; 113:345–357. [PubMed: 10357459]
- Pitkänen A, Amaral DG. The distribution of GABAergic cells, fibers, and terminals in the monkey amygdaloid complex: an immunohistochemical and in situ hybridization study. *J. Neurosci.* 1994; 14:2200–2224. [PubMed: 8158266]
- Raper J, Bachevalier J, Wallen K, Sanchez M. Neonatal amygdala lesions alter basal cortisol levels in infant rhesus monkeys. *Psychoneuroendocrinology.* 2013a; 38:818–829. [PubMed: 23159012]
- Raper J, Wallen K, Sanchez M, Stephens SBZ, Henry A, Villareal T, Bachevalier J. Sex-dependent role of the amygdala in the development of emotional reactivity to threatening stimuli in infant rhesus monkeys. *Horm. Behav.* 2013b; 63:646–658. [PubMed: 23380162]
- Resko JA, Goy RW, Robinson JA, Norman RL. The pubescent rhesus monkey: some characteristics of the menstrual cycle. *Biol. Reprod.* 1982; 27:354–361. [PubMed: 7126735]
- Rosvold HE, Mirsky AF, Pribram KH. Influence of amygdalectomy on social behavior in monkeys. *J. Comp. Physiol. Psychol.* 1954; 47:173–178. [PubMed: 13163250]
- Sokal RR, Braumann CA. Significance tests for coefficients of variation and variability profiles. *Syst. Zool.* 1980; 29:50–66.
- Terasawa E, Kurian JR, Keen KL, Shiel NA, Colman RJ, Capuano SV. Body weight impact on puberty: effects of high-calorie diet on puberty onset in female rhesus monkeys. *Endocrinology.* 2012; 153:1696–1705. [PubMed: 22315448]

- Thompson C, Schwartzbaum JS, Harlow HF. Development of social fear after amygdalectomy in infant rhesus monkeys. *Physiol. Behav.* 1969; 4:249–254.
- Wallen K, Lloyd EA. Clitoral variability compared with penile variability supports nonadaptation of female orgasm. *Evol. Dev.* 2008; 10:1–2. [PubMed: 18184351]
- Wierson M, Long PJ, Forehand RL. Toward a new understanding of early menarche: the role of environmental stress in pubertal timing. *Adolescence.* 1993; 28:913–924. [PubMed: 8266844]
- Wilson ME, Bounar S, Godfrey J, Michopoulos V, Higgins M, Sanchez M. Social and emotional predictors of the tempo of puberty in female rhesus monkeys. *Psychoneuroendocrinology.* 2013; 38:67–83. [PubMed: 22658962]
- Wilson ME, Gordon TP. Season determines timing of first ovulation in rhesus monkeys (*Macaca mulatta*) housed outdoors. *J. Reprod. Fertil.* 1989; 86:435–444. [PubMed: 2760874]
- Wilson ME, Gordon TP, Collins DC. Ontogeny of luteinizing hormone secretion and first ovulation in seasonal breeding rhesus monkeys. *Endocrinology.* 1986; 118:293–301. [PubMed: 3940852]
- Wilson ME, Gordon TP, Rudman CG, Tanner JM. Effects of a natural versus artificial environment on the tempo of maturation in female rhesus monkeys. *Endocrinology.* 1988; 123:2653–2661. [PubMed: 3197640]
- Wilson ME, Kinkead B. Gene–environment interactions, not neonatal growth hormone deficiency, time puberty in female rhesus monkeys. *Biol. Reprod.* 2008; 78:736–743. [PubMed: 18160679]
- Zehr JL, Van Meter PE, Wallen K. 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.* 2005; 72:1087–1094. [PubMed: 15625235]

Figure 1.

Coronal sections through the anterior (top) to posterior (bottom) extent of the amygdala.

Left column: T1-weighted MR images through the amygdala at one month of age in one sham-operated control (Neo-C-F4). Middle column: FLAIR images illustrating the location and extent of hypersignals (black arrows) within the amygdala in a representative case with neonatal amygdala lesion (Neo-A-F4). Right column: T1-weighted MR images through the amygdala at 1-year post-surgery in case Neo-A-F4 illustrating the enlargement of ventricles (white arrows) resulting from amygdala volume reduction.

Figure 2.

(a) Age (days) at menarche and first ovulation and (b) duration (days) of adolescent sterility for amygdala-lesioned (solid circles) and control females (open circles). Gray circles (solid = Neo-A; open = Neo-C) indicate the females that were peer-housed at 1.5 years of age. 1.5 years of age = 548 days; 2.5 years of age = 913 days, 3.5 years of age = 1278 days. * indicates significant difference ($p < .05$) between Neo-A and Neo-C females.

Figure 3.

Relationship between mean body weight at 1.5 years of age and age at menarche (a), age at first ovulation (b), and duration of adolescent sterility (c) for control females (open circles) and amygdala-lesioned females (solid circles). X indicates the Neo-A outlier excluded from analysis. * indicates a significant relationship for that neonatal treatment group.

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. (2013a). First ovulation data on Neo-A-F6 were not available.

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	$r(7) = -.82, p = .023^*$	$r(7) = -.34, p = .455$	$r(7) = -.62, p = .142$	$r(7) = -.53, p = .222$
Age at first ovulation	$r(6) = -.50, p = .309$	$r(6) = .85, p = .031^*$	$r(6) = .62, p = .191$	$r(6) = .66, p = .154$

* Significant relationship, $p < .05$.

Table 2

Regression and correlation statistics for menarche, first ovulation, and adolescent sterility with respect to neonatal treatment (Neo-A vs. Neo-C), juvenile social rank, and juvenile body weight.

	Menarche	First ovulation	Adolescent sterility (duration)
Neonatal treatment	$\beta = -.60, t(14) = -2.81, p = .014^*$; $R^2 = .36, F(1,15) = 7.88, p = .014$	$\beta = -.54, t(13) = -2.33, p = .037^*$; $R^2 = .30, F(1,14) = 5.43, p = .037$	$\beta = -.20, t(13) = -0.74, p = .474$; $R^2 = .04, F(1,14) = 0.54, p = .474$
Juvenile social rank	$\beta = .23, t(14) = 0.87, p = .399$; $R^2 = .05, F(1,15) = 0.76, p = .399$	$\beta = .32, t(13) = 1.23, p = .24$; $R^2 = .10, F(1,14) = 1.52, p = .24$	$\beta = .19, t(13) = 0.70, p = .494$; $R^2 = .04, F(1,14) = 0.50, p = .494$
Neo-A	$r(7) = .82, p = .023^*$	$r(6) = .57, p = .235$	$r(6) = .43, p = .392$
Neo-C	$r(9) = .27, p = .481$	$r(9) = .33, p = .379$	$r(9) = .06, p = .886$
Neonatal treatment, Juvenile body weight	$\beta = .45, t(13) = 2.17, p = .05$; $R^2 = .53, F(2,15) = 7.32, p = .007$	$\beta = -.35, t(12) = -1.44, p = .175$; $R^2 = .40, F(2,14) = 3.96, p = .048$	$\beta = -.64, t(12) = -2.63, p = .022^*$; $R^2 = .39, F(2,14) = 3.87, p = .051$
Neo-A	$r(6) = .23, p = .660$	$r(5) = .99, p = .002^{**}$	$r(5) = .95, p = .012^*$
Neo-C	$r(9) = .59, p = .092$	$r(9) = -.62, p = .074$	$r(9) = -.88, p = .002^{**}$

*
 $p < .05$.

**
 $p < .01$.