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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 amygdalectomy 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.

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

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parental divorce or an unfamiliar male living in the household accelerates menarche (Moffit 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 Kinkead, 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 matrilines, excluding the highest- and lowest-ranking matrilines, so that females had comparable social contexts, with all females having matrilines 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 × 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 × 256 matrix) were taken at 1 mm