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INTEREST IN INFANTS BY FEMALE RHEBUS MONKEYS WITH NEONATAL LESIONS OF THE AMYGDALA OR HIPPOCAMPUS

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Abstract

Previous research in our laboratory has shown that damage to the amygdala in neonatal rhesus monkeys profoundly alters behaviors associated with fear processing, while leaving many aspects of social development intact. Little is known, however, about the impact of neonatal lesions of the amygdala on later developing aspects of social behavior. A well-defined phenomenon in the development of young female rhesus monkeys is an intense interest in infants that is typically characterized by initiating proximity or attempting to hold them. The extent to which young females are interested in infants may have important consequences for the development of species-typical maternal behavior. Here we report the results of a study that was designed to assess interest in infants by female rhesus monkeys that received neonatal lesions to the amygdala, hippocampus or a sham surgical procedure. Subjects were first paired with pregnant “stimulus” females to assess social interactions with them prior to the birth of the infants. There were few behavioral differences between lesion groups when interacting with the pregnant females. However, following the birth of the infants, the amygdala-lesioned females showed significantly less interest in the infants than did control or hippocampus-lesioned females. They directed fewer affiliative vocalizations and facial expressions to the mother-infant pair compared to the hippocampus-lesioned and control females. These findings suggest that neonatal damage to the amygdala, but not the hippocampus, impairs important precursors of non-human primate maternal behavior.

Keywords

Macaca mulatta; social behavior; lesion; maternal behavior; development

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INTRODUCTION

Damage to the amygdala in neonatal rhesus monkeys profoundly alters behaviors associated with fear, while leaving fundamental aspects of social behavior intact (Bauman et al., 2004a, b). During the first year of life, for example, amygdala-lesioned subjects displayed heightened social fear when paired with familiar or novel conspecifics, and a decrease in fear of novel, inanimate objects. Despite these abnormalities in fear processing, all subjects were capable of producing a wide repertoire of social behaviors, were motivated to interact with peers in a variety of social contexts, and responded appropriately to social signals from conspecifics. We have concluded from this series of studies that early damage to the amygdala has minimal impact on the development of the basic components of social behavior, such as the ability to live successfully in a social group. This does not rule out the possibility that there might be consequences for later aspects of social development, such as maternal behavior.

In the present study, we evaluated whether neonatal damage to the amygdala or hippocampus impacts species-typical interest in infant conspecifics. Interest in infants is a common phenomenon in the development of many primate species (Lancaster, 1971, Fairbanks, 1990, Maestripieri, 1994a, Manson, 1999, Silk, 1999, Slater et al., 2007). In Old World monkeys, infants are an attractive stimulus for other group members, especially immature females (Rowell et al., 1964, Maestripieri, 1994a, Waitt et al., 2007). Female rhesus monkeys begin to show interest in infants within the first year of life, before the onset of puberty, and engage in more handling of infants than do males during their juvenile period (Chamove et al., 1967, Lovejoy and Wallen, 1988). Previous research has shown that specific types of vocalizations (e.g., girneys), facial expressions (e.g., lipsmacks) and handling behaviors (i.e., touches and embraces) are generated with greater frequency by young female primates when they are in the presence of infant conspecifics (Rowell et al., 1964, Chamove et al., 1967, Spencer-Booth, 1968, Gibber and Goy, 1985, Lovejoy and Wallen, 1988, Whitham et al., 2007). Though the functional significance of this behavioral phenomenon is unclear, it may be related to the development of future parenting skills (Quiatt, 1979, Meaney et al., 1990, Silk, 1999).

Converging lines of evidence from human and animal studies indicate that the amygdala may play a role in the development of appropriate responsiveness to infants. Human mothers, for example, show increased activation in the amygdala when listening to infant vocalizations (Sander et al., 2007) and when viewing pictures of their own infants (Ranote et al., 2004). Moreover, amygdala lesions in rodents and non-human primates alter maternal responsiveness (Kling, 1972, Steklis and Kling, 1985, Numan et al., 1993, Mann and Babb, 2004). While previous non-human primate lesion studies implicated temporal lobe areas as significantly contributing to the expression of appropriate maternal behavior, issues concerning the extent of lesion damage, previous maternal experience of subjects prior to the lesion and limited quantitative behavioral data complicate the interpretation of these data.

The present study was designed to assess qualitatively and quantitatively the interest in infants displayed by a cohort of nulliparous rhesus female monkeys that received lesions to the amygdala, hippocampus or a sham surgical procedure. Behavioral data were collected while subjects were free to interact with a multiparous female before and after the birth of her infant. These studies were thus designed to determine whether the neonatal lesions impacted the development of species-typical responsiveness to infant conspecifics.

METHODS

All experimental procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and developed through consultation with the veterinary staff at the California National Primate Research Center (CNPRC). All

protocols were also approved by the University of California, Davis, Institutional Animal Care and Use Committee.

Subjects and Living Conditions

This experiment was conducted with the female subjects from a larger, mixed-sex population of monkeys that received neonatal lesions of the amygdala, the hippocampus, or a sham surgical procedure at two weeks of age (Bauman et al., 2004a, b). Female subjects ($n = 14$) were approximately 3.5 years of age at the onset of these experiments. All were nulliparous and had no prior experience interacting with younger infant conspecifics as they aged. A brief summary of rearing/housing conditions is provided below. Subjects were randomly assigned to one of three lesion conditions: bilateral amygdala lesions (five females), bilateral hippocampus lesions (five females) or sham-operated controls (four females).

Females used in the present experiment were part of larger study which included a total of twenty-four infant rhesus monkeys (*Macaca mulatta*) naturally born of multiparous mothers, and randomly assigned to one of three lesion conditions: bilateral amygdala lesions (five females, three males), bilateral hippocampus lesions (five females, three males) or sham-operated controls (four females, four males). All surgeries were performed at 12–16 days after birth. The infants were returned to their mothers following surgery and provided daily access to a socialization group consisting of six mother-infant pairs and one adult male. The four socialization groups were each composed of two amygdala-lesioned infants and their mothers, two hippocampus-lesioned infants and their mothers, and two sham-operated infants and their mothers. When the youngest subject within a socialization group reached six months of age, the infants were permanently separated from their mothers, but otherwise continued to experience the same housing and group socialization regimen. At approximately one year of age, subjects became permanently housed socially (24 hours per day) with their original socialization cohort in a (2.13 m $W \times$ 3.35 m $D \times$ 2.44 m H) chain link enclosure. At approximately three years of age, each cohort was relocated to an outdoor enclosure (4.9 m $W \times$ 4.3 m $D \times$ 2.4 m H) and had lived there approximately two weeks at the onset of this experiment. It is important to note that all subjects were reared by their mothers and provided daily 3-hr access to a social cohort to approximate the features of macaque social organization that appear necessary for the development of species-typical behavior. While all subjects interacted with other age-matched peers in their rearing cohorts, membership in these groups remained unchanged throughout their development. Thus, as they aged, subjects did not have the opportunity to interact with other mother-infant pairs or younger conspecifics at any point prior to the onset of these experiments.

Surgical Procedures

The surgical procedures are summarized below and are described in detail in previous publications (Bauman et al., 2004a, c). On the day of surgery, the infants were initially anesthetized with ketamine hydrochloride (15 mg/kg i.m.) and medetomidine (30 μ g/kg), and placed in a magnetic resonance imaging (MRI)-compatible stereotaxic apparatus (Crist Instruments Co., Inc., Damascus, MD). The infant's brain was imaged using a General Electric 1.5 T Gyroscan magnet; 1.0 mm thick sections were taken using a T1-weighted Inversion Recovery Pulse sequence (TR = 21, TE = 7.9, NEX 3, FOV = 16cm, Matrix, 256 $\times$ 256). From these images, we determined the location of the amygdala or hippocampus and calculated the coordinates for the ibotenic acid injections. Infants were ventilated and vital signs monitored throughout the surgery. A stable level of anesthesia was maintained using a combination of isoflurane (1.0%, varied as needed to maintain an adequate level of anesthesia) and intravenous infusion of fentanyl (7–10 μ g/kg/hour). Following a midline incision, the skin was laterally displaced to expose the skull, two craniotomies were made over the amygdala or the hippocampus, depending on the pre-determined lesion condition, and the dura was reflected

to expose the surface of the brain. Ibotenic acid (IBO, Biosearch Technologies Inc., 10 mg/ml in 0.1 M phosphate buffered saline) was injected simultaneously bilaterally into the amygdala or hippocampus using 10 μ l Hamilton syringes (26 gauge beveled needles) at a rate of 0.2 μ l / min. Sham-operated controls underwent the same pre-surgical preparations, received a midline incision and the skull was exposed. Control subjects were maintained under anesthesia for the average duration of the lesion surgeries and the fascia and skin were sutured in two separate layers. Following the surgical procedure, all infants were monitored by a veterinarian and returned to their mothers once they were fully alert (generally within 24 hours).

Lesion Analysis

We obtained T2-weighted magnetic resonance (MR) images ten days after surgery to examine the extent of the edema associated with the lesion. The hyperintense T2-weighted signal for each of the sixteen lesioned subjects was evaluated to confirm the general target and extent of the lesions (i.e., amygdala lesion sparing the hippocampus or hippocampus lesion sparing the amygdala). The animals brains were imaged using a General Electric 1.5 T Gyroscan magnet; 1.5 mm thick sections were taken using a T2 weighted Inversion Recovery Pulse sequence (TR = 4000, TE = 102, NEX 3, FOV = 16cm, Matrix, 256 $\times$ 256). Additional lesion confirmation was provided by T1-weighted MR images obtained at approximately four years of age. The animals' brains were scanned using a General Electric 1.5T Signa MRI system; 1mm thick sections were taken using a T1 weighted 3D axial spoiled gradient (SPGR) sequence (TR = 22.0ms, TE = 7.9ms, NEX 3, FOV = 16cm, Matrix, 256 $\times$ 256).

Study Design

Observations took place in a large indoor chain-link enclosure (2.13m *W* $\times$ 3.35m *D* $\times$ 2.44m *H*). Testing was always conducted between 11:00am-1:00pm. Female subjects were relocated indoors for testing and then returned to their outdoor social groups at the end of each test session. Testing took place in the same room and enclosures in which the subjects had been previously housed. Thus, all subjects were familiar with the environment and enclosure set-up, which helped to minimize any stress associated with transport and handling procedures.

Testing was conducted in two phases (termed pre-birth and post-birth dyads) with a one-month interval between phases.

Pre-birth Dyads—Each female subject was introduced to one of three pregnant, adult female stimulus monkeys. To minimize disruptive emotional reactions such as fear or aggression associated with introductions of unfamiliar partners, each female subject was paired with the stimulus female prior to her giving birth (pre-birth dyads) for 20 minutes on four separate occasions. Behavioral data were collected to assess basic social interactions between the female subject and stimulus female before the birth of her infant. Data collection consisted of observing each subject for two consecutive 10-min focal samples during each 20-min dyad. Thus, a total of eight 10-minute focal samples were collected for each female subject.

Females from a particular cohort were individually paired with the same stimulus female (i.e., Cohort #1 females met Stimulus female A, Cohort #2 females met Stimulus female B, and Cohort #3 & #4 females met Stimulus female C). The stimulus females ranged in age from 10–12 years, all were multiparous and were in their third trimester of pregnancy (range of 127–151 gestational days) at the onset of testing. The stimulus infants for the post-birth phase of testing consisted of two females (Stimulus Infant A & C) and one male (Stimulus Infant B).

Post-birth Dyads—Approximately 2 weeks after the infants were born subjects were re-introduced to the same stimulus female they met in the pre-birth dyads with her infant (the post-birth dyads). Each female subject and mother-infant pair was together for 30 minutes once

per week for 12 consecutive weeks. Behavioral data collected during each dyad was based on three consecutive 10-min focal samples on each female subject each week for a total of thirty-six samples.

In addition, when a stimulus infant reached four weeks of age, the frequency and duration of time it spent in contact, proximity, and out of arm's reach in reference to its mother was simultaneously recorded during each dyad. A total of twenty-seven, 10-minute focal samples were collected for each stimulus infant. These data were used to assess whether the degree to which stimulus females restricted their infants varied in relation to the lesion condition of the female subject with which they were paired.

Behavioral Ethogram

Behavioral data were collected with The Observer software (Noldus, 1991) by trained observers demonstrating an inter-observer reliability $\geq 90\%$ (agreements/[agreements + disagreements] X 100). The frequency and duration of behaviors were collected using a catalog of behaviors typical of this species with specific behaviors included in order to assess interest in infant conspecifics (Herman et al., 2003, Whitham et al., 2007) (see Table 1). Behaviors commonly elicited from rhesus females by the presence of infants were included, such as specific forms of vocalizations (grunts and girneys), facial expressions (lipsmacks), as well as physical contact behaviors (genital inspection, groom, non-aggressive contact, touch, restrain, and grapple play). Observers recorded the directionality of the behavior (initiated by the focal subject or received by the stimulus mother or infant) and the identity of the recipient of the behavior (either focal subject, mother, infant, or mother-infant pair). In addition, we have adopted a scoring method that requires a behavioral event cease for 3 seconds before it can be re-scored. Likewise, a behavioral state must maintain for at least 3 seconds before it can be scored. When a behavior is recorded, a time-stamp is also recorded. In addition, a second observer recorded for each stimulus infant the frequency and duration of time it spent in contact, proximity, and out of arms' reach of its mother during each dyad (Table 2).

Statistical Analysis

Analyses of variance (ANOVAs) followed by Fisher's protected least significant difference (PLSD) post-hoc tests (with a significance level of $p < 0.05$) were used for data analyses. The frequency and duration of behaviors was averaged and then analyzed using a series of one-way ANOVAs with lesion condition used as the between-subjects factor. Co-variate analyses (ANCOVA) were applied to the data whenever appropriate. Repeated measures ANOVAs were conducted to assess the effect of test day and test condition (pre vs. post-birth dyads) on the expression of behaviors. For pre-birth dyads, data were analyzed with lesion condition as the between-subject factor and test day (4) as the within-subject factor. For post-birth dyads, data were divided into three 4-week blocks, with lesion condition as the between-subject factor and block (3) as the within-subject factor. To evaluate any effects the presence of the infants had on the behavioral responses by the female subjects, data from the first four weeks of post-birth dyads (i.e., Block 1) were used to compare with the pre-birth dyads. Two-factor ANOVA (Stimulus Infant X Lesion Status of Female Subject) was used to analyze whether the stimulus infants' frequency and duration of time spent on and off their mothers was dependent on the lesion status (amygdala-or hippocampus-lesioned or control) of the female with which the infants were paired.

Data were analyzed and are presented as the average frequency or duration (in seconds) per 10-minute focal observation. We analyzed the frequency and duration for each behavior for effect of lesion in both the pre-and post-birth dyad conditions. We then grouped behaviors (e.g., calculated the mean frequency of a group of behaviors) into broad categories (affiliative vocalizations, physical contact, fearful, and aggressive) based on previous descriptions in the

literature (Table 1). Although observers recorded each behavior initiated by the focal subject as being directed to either the mother, infant, or mother/infant unit, when infants were in contact with their mothers it was often difficult to determine whether facial expressions or vocalizations were being directed by the focal subject to the mother, infant, or both. Therefore, the recipient (mother, infant or mother/infant unit) of facial expressions (i.e., lipsmack, fear grimace, threat) and vocalizations (i.e., girney, grunt, scream, bark) were grouped into a single recipient category for analysis and is referred to in the text as mother-infant pair.

RESULTS

Magnetic Resonance Imaging and Histological Evaluation of Lesions

T2-weighted images of coronal sections are illustrated in previous publications, providing substantial reassurance that the ibotenic acid was injected and was focused in the amygdaloid complex or hippocampal formation (Bauman et al., 2004a, c). The extent of the targeted lesion was confirmed in one amygdala-lesioned subject that died due to an unrelated illness and whose brain was subjected to histological evaluation of the lesion (see Figure 2 in Bauman et al. 2004b). Analysis of a second series of structural MRIs performed when the subjects were approximately 4 years of age provided additional confirmation of the lesions (Bauman et al., 2006). Qualitative assessment of the lesion extent revealed that all eight amygdala-lesioned subjects demonstrated substantial bilateral damage to the amygdaloid complex, as indicated by clear shrinkage of the amygdala and/or expansion of the ventricles into space formerly occupied by the amygdala. If there was any sparing of amygdala tissue, it was limited to the most caudal aspects of the amygdala, perhaps including the central nucleus. Analysis of the hippocampus lesions revealed nearly complete bilateral damage for all cases, with minimal sparing of the extreme rostral and caudal portions (Lavenex et al., 2007). These qualitative observations of the lesion extent are further supported by recent PET neuroimaging of these subjects (Machado et al., 2008).

Pre-birth Dyad Findings

Differences between lesion groups in behaviors initiated by the experimental subjects or received from the stimulus females during the pre-birth dyads were few and most were not statistically significant (Table 3).

Fear Behaviors—The effects of lesion on specific fear behaviors directed from the female subjects to the stimulus females were not significant (i.e., avoid: $F_{(2,11)} = 3.340$, $p = 0.0735$; fear grimace: $F_{(2,11)} = 1.214$, $p = 0.3338$; flee: $F_{(2,11)} = 0.538$, $p = 0.5987$; freeze: $F_{(2,11)} = 1.310$, $p = 0.3089$; or scream: $F_{(2,11)} = 0.877$, $p = 0.4431$). Given that individual fear behaviors did not reveal an effect of lesion, we simplified the analysis by summing and analyzing all behaviors associated with the initiation of fear.

Experimental groups did not differ in their total expression of these behaviors ($F_{(2,11)} = 1.234$, $p = 0.3285$). These findings demonstrate that amygdala-lesioned subjects were not differentially fearful of the stimulus females when compared with hippocampus-lesioned and control subjects.

Threat Response by the Stimulus Females—Threats by the stimulus females toward the subject females did not differ reliably between lesion groups ($F_{(2,11)} = 1.643$, $p = 0.2375$), suggesting that the stimulus females did not distinguish between the lesion status of the subject females. The overall incidence for other aggressive behaviors (contact aggression, chase, and displacement) was very low and did not differentiate lesion groups.

Social Interest—To provide an indication of how socially interested the focal subjects were of the stimulus females, behaviors such as approach, follow, proximity, contact, and grooming were analyzed. Lesion group differences for these behaviors were modest. In general, the hippocampus-lesioned females engaged more with the novel, stimulus females than did amygdala-lesioned and control females. They approached the stimulus females more than amygdala-lesioned and control females ($F_{(2,11)} = 23.529, p = 0.0001; p < .0001$ and $p = 0.0031$, respectively); while control females approached the stimulus females more than amygdala-lesioned females ($p = 0.0219$). Hippocampus-lesioned females also initiated more instances of proximity to the stimulus females than amygdala-lesioned females ($F_{(2,11)} = 7.968, p = 0.0073; p = 0.0021$). Control females did not differ significantly in their frequency of proximity to the stimulus females when compared to the other two groups. The instances of follow, contact or groom initiated to the stimulus females were low and did not reveal an effect of lesion (Follow: $F_{(2,11)} = 1.303, p = 0.3106$; Contact: $F_{(2,11)} = 2.080, p = 0.1713$; Groom: $F_{(2,11)} = 0.745, p = 0.4971$).

It is important to note that lesion groups did not differ significantly in their production of affiliative vocalizations, such as grunts and girneys, (mean frequency per 10-min sample: control group = 1.158 ± 0.965 ; hippocampus group = 1.526 ± 0.697 ; amygdala group = 2.404 ± 2.579) and lipsmacks (mean frequency per 10-min sample: control group = 0.470 ± 0.694 ; hippocampus group = 0.552 ± 0.548 ; amygdala group = 1.352 ± 1.637) during the pre-birth phase of data collection.

None of the behaviors reported above revealed a significant effect of test day (1–4) or a significant interaction of test day and lesion condition.

Post-birth Dyad Findings

The pattern of behavioral findings tended to remain consistent between the two test conditions with the exception of a few key behaviors known to be elicited more frequently in the presence of infants (Table 3).

Fear Behaviors—As reported for the pre-birth dyads, lesion groups did not differ reliably on fear behaviors directed to the mother-infant pair (i.e., avoid: $F_{(2,11)} = 2.280, p = 0.1485$; fear grimace: $F_{(2,11)} = 0.452, p = 0.6478$; flee: $F_{(2,11)} = 1.065, p = 0.3777$; freeze: $F_{(2,11)} = 0.611, p = 0.5602$; or scream: $F_{(2,11)} = 0.396, p = 0.6822$). Similarly, the effect of lesion condition on the combined fear behaviors was not significant (i.e., avoid, fear grimace, freeze, flee, scream) ($F_{(2,11)} = 0.839, p = 0.4581$).

Threat Response by the Stimulus Females—Lesion condition also had no effect on threats received from the stimulus females ($F_{(2,11)} = 2.285, p = 0.1480$). This suggests that the lesion status of the subjects did not prompt differential threat rates from the stimulus females.

Social Interest—The hippocampus-lesioned females continued to spend more time in proximity to the mother-infant pair during the post-birth dyads ($F_{(2,11)} = 2.217, p = 0.0384$) than did amygdala-lesioned females ($p = 0.0128$), though frequency of proximity failed to distinguish lesion groups ($F_{(2,11)} = 1.425, p = 0.2816$). Control females did not differ significantly in time spent in proximity to the mother-infant pair when compared to the other two groups. There were no consistent appreciable differences between the groups for the remaining behaviors associated with social interest, such as approach ($F_{(2,11)} = 1.540, p = 0.2572$), follow ($F_{(2,11)} = 2.355, p = 0.1409$), contact ($F_{(2,11)} = 2.415, p = 0.1351$) and groom ($F_{(2,11)} = 1.836, p = 0.2050$).

Infant Interest—Although each of the subject females was encountering an infant for the first time in the post-birth dyads, lesion groups differed substantially on behaviors specific to infant-related responsiveness in rhesus monkeys. On average, amygdala-lesioned females directed less than half as many affiliative vocalizations (grunts and girneys) ($F_{(2,11)} = 9.591$, $p = 0.0039$) toward the mother-infant pair than did control and hippocampus-lesioned females ($p = 0.0012$ and $p = 0.0204$, respectively) (Figure 1). They also produced many fewer lipsmacks at the mother-infant pair ($F_{(2,11)} = 5.561$, $p = 0.0214$) than either control or hippocampus-lesioned females ($p = 0.0084$ and $p = 0.0399$, respectively) (Figure 2). The mothers did not appear to distinguish between the lesion status of the subject females as the frequency and duration of time that the infants spent off their mothers was not dependent on the lesion status of the female with which they were paired ($F_{(2,4)} = 3.294$, $p = 0.1116$ and $F_{(2,4)} = 3.279$, $p = 0.1124$, respectively).

Given that hippocampus-lesioned females spent significantly more time in proximity to the mother-infant pair than did amygdala-lesioned subjects, it was important to assess whether differences in proximity influenced the production of affiliative vocalizations and lipsmacks directed toward the mother-infant pair. To assess this, time spent in proximity was added as a covariate in the two ANOVAs. The duration of proximity was not a significant covariate for either affiliative vocalizations ($F_{(1,10)} = 1.711$, $p = 0.2201$) or lipsmacks ($F_{(1,10)} = 2.703$, $p = 0.1312$). Furthermore, adding the duration of proximity did not influence the overall pattern of effects; affiliative vocalizations and lipsmacks remained significantly different between lesion groups ($F_{(2,10)} = 10.845$, $p = 0.0031$, $F_{(2,10)} = 7.733$, $p = 0.0093$) when the duration of proximity was normalized across lesion groups. Therefore, controlling for time spent in proximity does not appear to influence the pattern of findings as lesion condition remained a significant predictor of affiliative vocalizations and lipsmacks. It is also important to note that time spent in proximity during the post-birth dyads only statistically differed between hippocampus- and amygdala-lesioned females. The time that control females spent in proximity to the mother-infant pair fell between that of hippocampus- and amygdala-lesioned animals even though on average, they produced more vocalizations and lipsmacks than the other two groups (refer to Table 3). This is further evidence that the initiation of affiliative vocalizations and lipsmacks were not dependent on being in proximity.

There was no effect of lesion group on the frequency of infant contact behaviors (i.e., touch, groom, play, ano-genital inspection, and non-aggressive contact) ($F_{(2,11)} = 1.365$, $p = 0.2954$). The failure to detect differences between lesion groups likely reflects, at least in part, differences among the stimulus females' in maternal styles, particularly in terms of the degree of restriction the stimulus females imposed on their infants. One indication of this difference was the amount of time infants spent out of their mother's arm reach. The least restricted infant (Stimulus Infant A) differed significantly from the other two infants in time spent out of arms' reach of its mother ($F_{(2,11)} = 6.067$, $p = 0.0168$) (Stimulus Infant C: $p = 0.0060$ and Stimulus Infant B: $p = 0.0462$, respectively) (mean duration per 10-min sample: Stimulus Infant A = $79.813 \text{ s} \pm 52.955$; Stimulus Infant B = $24.297 \text{ s} \pm 20.092$; Stimulus Infant C = $10.348 \text{ s} \pm 10.686$). Another indication which highlights this difference was the frequency of threats initiated by the mothers. Stimulus Mother A initiated significantly less threats toward the subject females than the other two mothers ($p = 0.0096$ and $p = 0.0047$, respectively). Stimulus Mother A averaged less than one threat per sample while Stimulus Mother B averaged five threats per sample, and Stimulus Mother C averaged more than nine threats per sample. A presumed consequence of these differences is that access to the infants was not comparable, resulting in considerable variation between subject females in their frequencies of physical contact of the infants. Indeed, the two control females that interacted with the more restricted Stimulus Infant C never physically contacted the infant and the two hippocampus-lesioned females that interacted with this same infant averaged less than one physical contact episode. In contrast, the single control and two hippocampus-lesioned females tested with Stimulus

Infant A averaged more than 2 acts of physical contact per 10-min observation period, with touches to the infant accounting for >50% of total acts of physical contact. We observed almost no attempts from the five amygdala-lesioned subjects to contact any of the infants, regardless of whether the amygdala-lesioned subjects had the opportunity to engage in such contact. Of these five amygdala-lesioned female subjects, only one had a single episode of physical contact with an infant.

None of the behaviors reported above revealed a significant effect of test block (1–3) or a significant interaction of test block and lesion condition.

Comparison of Pre- and Post-birth Dyads

Repeated Measures ANOVAs were conducted in order to assess the impact of the presence of the infants on the subjects' behaviors using testing condition (pre-birth v. post-birth) as the repeated factor and lesion condition (amygdala, hippocampus, control) as the between subjects factor. Results revealed a few small but instructive differences between lesion groups (Table 4).

Fear Behaviors—Fear behaviors (avoid, fear grimace, flee, freeze, scream, or fear behaviors combined, $p > 0.10$ for all analyses) did not differ significantly between test condition or subject groups.

Threat Response by the Stimulus Females—The frequency of threats received from the stimulus females revealed a marginally significant condition effect ($F_{(1,11)} = 4.532, p = 0.0567$). They tended to threaten female subjects more frequently in the post-birth condition, though the follow-up ANOVA failed to reach statistical significance ($F_{(1,26)} = 3.394, p = 0.0769$).

Social Interest—The frequency of approaches ($F_{(1,11)} = 2.538, p = 0.1395$) and proximity ($F_{(1,11)} = 0.760, p = 0.4019$), and the interaction of lesion and test condition was not significant for either behavior ($F_{(2,11)} = 0.520, p = 0.6085, F_{(2,11)} = 0.339, p = 0.7199$, respectively).

Infant Interest—The frequency of affiliative vocalizations (i.e., grunts and girneys) and lipsmacks showed a large and significant increase in the presence of the infants ($F_{(1,11)} = 63.527, p = <.0001, F_{(1,11)} = 27.249, p = 0.0003$, respectively). Affiliative vocalizations and lipsmacks were more frequent during the initial encounter with the infants (Block 1, post-birth) than the pre-birth condition ($F_{(1,26)} = 32.643, p = <.0001, F_{(1,26)} = 14.192, p = 0.0009$, respectively). Not all experimental groups, however, increased their frequency of these affiliative signals in the presence of the infants as an interaction between lesion status and test condition was found to be statistically significant for both behaviors ($F_{(2,11)} = 5.563, p = 0.0214, F_{(2,11)} = 4.570, p = 0.0359$, respectively). Control and hippocampus-lesioned females produced more affiliative vocalizations during Block 1 of post-birth dyads than the pre-birth dyads ($F_{(1,6)} = 35.081, p = 0.0010, F_{(1,8)} = 31.846, p = 0.0005$, respectively). Likewise, control and hippocampus-lesioned females produced more lipsmacks in the presence of the infants compared to the pre-birth dyad condition ($F_{(1,6)} = 6.253, p = 0.0465, F_{(1,8)} = 15.261, p = 0.0045$, respectively). Amygdala-lesioned females, however, did not appreciably differ in their production of these affiliative signals in the presence of the infants when compared to the pre-birth condition (Affiliative vocalizations: $F_{(1,8)} = 1.943, p = 0.2009$ and Lipsmacks: $F_{(1,8)} = 0.278, p = 0.6126$).

DISCUSSION

The present study provides evidence that monkeys that received lesions to the amygdala in early infancy displayed significantly decreased species-typical interest in infants, as compared to monkeys that received lesions to the hippocampus or a control sham surgical procedure. Lesion groups differed very little in their reactions to the stimulus females prior to the birth of the infant. There were few differences between groups for the majority of behavioral measures assessed during the pre-birth dyads, including fear, aggressive, and affiliative behaviors. In contrast, during the post-birth dyads, groups differed substantially on behaviors known to be elicited more frequently in the presence of infants, such as lipsmacks and affiliative vocalizations. Given that the findings in the post-birth condition centered around signals that are characteristically displayed by rhesus females in the presence of infants, the differences between groups were almost certainly related to the presence of the infants and do not reflect a generalized difference in sociability per se. While infant handling behaviors, such as touching or carrying, failed to distinguish lesion groups in the present study, this is most likely due to the presence of the stimulus mothers and differences in their maternal styles. Previous studies which described infant handling behaviors as being the clearest manifestation of interest in infants by young female primates were usually documented in large, familial groups in which handling of infants was presumably more frequent and tolerated than in the present study. Taken together, our results indicate that the presence of the infants prompted the control and hippocampus-lesioned subjects to produce greater rates of vocalizations and lipsmacks than the amygdala-lesioned subjects.

It is notable that in contrast to our previous observations that the amygdala-lesioned subjects were more fearful during social interactions with conspecifics (Bauman et al., 2004a, b, Bauman et al., 2006), fear behaviors did not differentiate lesion groups in the present study. We observed no lesion group differences in the frequency of fear related behaviors directed toward the mother alone or the mother-infant pair in both dyad conditions. Nevertheless, the finding that amygdala-lesioned females initiated fewer approaches and bouts of proximity during the pre-birth dyads, and spent significantly less time in proximity to the mother-infant pair, suggests that they were more timid about engaging with the stimulus females socially, although discrete fear behaviors were absent. Thus, it remains uncertain if social fear somehow indirectly altered their ability to express interest in the mother-infant pair. In contrast, previous findings with these same hippocampus-lesioned subjects have consistently shown that they are indistinguishable from controls on the majority of behavioral assays we have used thus far (Bauman et al., 2004a, b, Bauman et al., 2006). We would also suggest that the hyperactivity previously reported in these hippocampus-lesioned subjects (Bauman et al., 2004a, Bauman et al., 2008) may have indirectly impacted their behavioral phenotype in the present study (i.e., an increase in approaches to the mother-infant pair when interacting in an open field test cage), though the precise impact of neonatal lesions of the hippocampus on social behavior will be more thoroughly examined in future publications.

Not only were the amygdala-lesioned females consistently less close to the mother-infant pair, they also produced fewer affiliative vocalizations (i.e., grunts and girneys) and lipsmacks toward them. Affiliative vocalizations such as grunts and girneys provide a clear index of interest in infants (Herman et al., 2003, Whitham et al., 2007). Importantly, these vocalizations provide a standard for measuring general interest in infants that is not necessarily related to the behavioral intention of the caller. Evidence from a recent study (Whitman et al., 2007) demonstrated that grunts and girneys in the presence of infants did not predict the behavioral actions of the caller (e.g., moving into proximity of the infant, handling the infant with care or roughness). Whitman and colleagues (2007) postulated that girneys and grunts directed to mother-infant pairs indicate a state of general arousal or interest of the caller induced by the presence of an infant, rather than information about the caller's intent or subsequent behavior.

This view is consistent with other theories of macaque vocalizations which suggest that they reflect information about the status of the caller (Owings and Morton, 1997, Owren and Rendall, 1997). Results of the present study showed that control and hippocampus-lesioned females, but not amygdala-lesioned females, produced more affiliative vocalizations when the infants were present even though most other behaviors were unchanged between the two test conditions. Consistent with Whitman's findings and theoretical framework, a reasonable interpretation of our data is that amygdala-lesioned females were less aroused by the presence of infants, and therefore less likely to produce the species-typical vocalizations that are normally evoked in the presence of infants.

The lack of affiliative behaviors by the amygdala-lesioned females does not appear to stem from an inability to produce them. Previous observations during the first two years of life revealed few differences between lesion groups in the production of affiliative behaviors during dyadic and group social interactions (Bauman et al., 2004a, c). Similarly, in the present studies we observed few differences between lesion groups in affiliative behaviors generated during the pre-birth dyads before the infants were present, nor in affiliative behaviors directed to only the mothers during the post-birth condition. It is also possible that the stimulus mothers may have responded differentially to the lesioned females, thereby influencing their expression of interest in the infants. This seems unlikely, however, as we found no differences among lesion groups in the frequency of threats or in affiliative behaviors the mothers produced.

Our findings are consistent with early non-human primate studies that indicated lesions of the temporal lobe structures had deleterious effects on maternal behavior. These early accounts described amygdalectomized female monkeys exhibiting maladaptive maternal behaviors toward their infants including physical abuse and neglect of their offspring (Kling, 1972, Steklis and Kling, 1985). Likewise, ablation lesions to the anterior temporal cortex also resulted in pathological maternal behavior by experienced rhesus mothers (Bucher et al., 1970). These early studies focused on how damage to the temporal lobe regions impacted maternal behavior, rather than affecting possible early precursors of this behavior, such as interest in infants.

A substantial literature based on rodent studies also confirms that the amygdala plays a critical role in facilitating species-specific components of maternal behavior. Interestingly, there are many differences between species in their behavioral patterns. Unlike juvenile female primates, for whom infants are a source of interest, nulliparous female rats typically respond with aggression and, or, aversion when presented with foster pups (Rosenblatt, 1967, Numan et al., 1993). A current model of maternal inhibition in rodents suggests that olfactory input to the amygdala activates hypothalamic regions, which in turn inhibits maternal behavior in nulliparous females (Sheehan et al., 2001). If any of the components of this inhibitory circuit are experimentally impaired, a rapid onset of maternal responsiveness results. For example, lesions of the amygdala (Fleming et al., 1980, Numan et al., 1993) or the anterior, ventromedial, and dorsal hypothalamic areas (Bridges et al., 1999, Mann and Babb, 2004) promotes maternal behavior in nulliparous rats when they are exposed to foster pups. Though there are many differences between rodents and non-human primates in their behavioral response toward infant conspecifics, nonetheless, damage to the amygdala results in a reversal of their usual species-specific behavioral responses toward infants. As such, results from rodent and primate lesion studies provide strong complementary evidence that the amygdala plays a critical role in facilitating species-specific components of maternal responsiveness.

A final issue to be addressed in the context of the findings presented here is the relationship between interest in infant conspecifics and maternal care of one's own offspring. Given that interest in infants is a predictable aspect of sexually differentiated behavior common to many non-human primate species, including humans (Lancaster, 1971, Fairbanks, 1990, Maestriperi and Pelka, 2002, Wallen, 2005), finding infants attractive may be largely determined by the

female genetic and hormonal predisposition for maternal care (Maestripieri, 1994b). Although the precise biological substrate of this difference is not understood, it is generally accepted that genetic and prenatal hormonal factors alter the developing nervous system, predisposing the fetus eventually to develop sex-specific behavioral traits (Wallen, 2005). Currently, it is not known which regions of the brain underlie this sexual differentiation of behavior. Our findings suggest that the amygdala may be one of the structures involved in the development of appropriate primate maternal responsiveness.

Though several studies have explored the adaptive significance of allomaternal behavior by young female primates toward infants (Quiatt, 1979, Meaney et al., 1990), the exact relationship between interest in infants and maternal behavior remains unresolved (Maestripieri, 1999). Historically, young females' interest in infants is thought to provide the substrate through which they gain experience necessary for mothering their own offspring (Lancaster, 1971). In this view, infant interest serves to engage young females in interaction with infants. By handling and attending to other females' infants, young females acquire the skills necessary for successful mothering. This hypothesis is supported by findings which show pre-pubescent females are often the most frequent handlers of infants and their handling and care-giving skills improve with practice (Meaney, 1990.) Prior parity is another factor that can intensify females' behavioral response toward infants. Multiparous rhesus monkeys often exhibit more appropriate care and retrieval of unfamiliar infants than do nulliparous females, suggesting that parity is a critical factor that can influence the propensity for providing care to infants (Holman and Goy, 1980, Gibber, 1986). Upon parturition, hormones, such as oxytocin, mediate a response which prime females to provide nurturance and care to their own offspring (Holman and Goy, 1980). Given that parturition, and its associated hormones, triggers rhesus females to exhibit an appropriate maternal response toward their offspring, it would be of interest to re-evaluate the amygdala-lesioned females' response to infants once they have given birth themselves. Thus, at present, we cannot unequivocally conclude that the reduced interest in infants by the amygdala-lesioned subjects suggests they would be impaired at rearing their own offspring.

In sum, rhesus female monkeys that sustained amygdala lesions as neonates, but not those with neonatal lesions to the hippocampus, demonstrate less species-typical interest in infants of other mothers later in life. Positioning this work within a broader picture of amygdala function is an experimental challenge for the future. One of the hallmark findings in lesion work in non-human primates and rodents is that removal of the amygdala compromises fear processing. In the present study, however, there were no observed differences in fear behaviors between amygdala-lesioned animals and the other two experimental groups. This raises the issue of whether the present finding is consistent with the more general finding of altered fear processing resulting from amygdala lesions and highlights a parallel and independent role of the amygdala in maternal behavior. It is important to note that we have since attempted to evaluate maternal behavior with the same cohort of amygdala lesioned animals. But, due to a number of technical difficulties, it has not been possible to conduct a rigorous evaluation of their ability to rear their own offspring. Although anecdotal accounts of amygdala-lesions in macaque monkeys have documented that the lesioned animals were abusive or neglectful of their own infants (Kling and Steklis, 1976), the extent to which they were interested in other infants was not assessed. It is known, however, that human females with bilateral lesions of the amygdala are fully capable of effectively rearing their own children (Adolphs et al., 1994, Amaral et al., 2003). It would be of some interest to revisit this topic with rhesus monkeys that have had early neonatal damage of the amygdala to clarify the functional significance of interest in infants, its relationship with maternal behavior and the neural mechanisms that regulate these behaviors.

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Figure 1. Frequency of Affiliative Vocalizations

Amygdala-lesioned females produced fewer affiliative vocalizations (grunts and girneys) toward the mother-infant pair during the post-birth condition than control or hippocampus-lesioned females. Each bar represents the average frequency per ten-minute observation period for the Pre- and Post-birth Dyads (error bars reflect the standard error of the mean). AMY = amygdala-lesioned subjects; CON = sham-operated subjects; HIP = hippocampus-lesioned subjects.

Figure 2. Frequency of Lipsmacks

Amygdala-lesioned females directed fewer lipsmacks at the mother-infant pair during the post-birth condition than control or hippocampus-lesioned females. Each bar represents the average frequency per ten-minute observation period for the Pre- and Post-birth Dyads (error bars reflect the standard error of the mean). AMY = amygdala-lesioned subjects; CON = sham-operated subjects; HIP = hippocampus-lesioned subjects.

Table 1

Social Behavior Ethogram

Behaviors	Description
Duration Behaviors	
Extended Groom	Picking or licking another animal's fur for > 3sec
Extended Contact	Physical, non-aggressive contact with another animal for > 3sec
Proximity	Within arm's reach of another animal for > 3sec
Extended Play	Play behavior lasting for > 3sec
Extended Aggression	Aggressive interactions lasting for > 3sec
Nonsocial Activity	Active behavior while out of proximity for > 3sec
Nonsocial Inactivity	Passive (head down/not exploring) out of proximity for > 3sec
Sleep Contact	Eyes closed while in contact with another for > 3sec
Sleep Proximity	Eyes closed while in proximity with another for > 3sec
Sleep Solo	Eyes closed while out of proximity for > 3sec
Frequency Behaviors	
SOCIAL INTEREST BEHAVIORS	
Approach	Intentional movement within arm's reach of another animal
Groom ^a	Examines, picks, or licks at another's fur or body part
Contact ^a	Physical, non-aggressive contact
Follow	Slow, deliberate movement after another animal lasting > 3sec
INFANT CONTACT BEHAVIORS	
Touch	Intentional touch of the infant with hand
Carry	Carries infant away from original location
Restrain	Physical restraint of infant such as holding a tail or limb
Anogenital Exploration	Oral, olfactory, or manual exploration of another's anogenital region
Social Play	Rough and tumble play, grappling
AFFILIATIVE VOCALIZATIONS	
Girney ^a	Quiet, nasal whine
Grunt ^a	Deep, muffled, low-intensity
FEAR BEHAVIORS	
Fear Grimace	Upper and lower lips retracted, exposing teeth
Avoid	Movement away due to the approach of another animal
Flee	Rapid movement away from another animal
Freeze	Animal does not move (crouching or sitting) for > 3sec
Scream	High-pitched screech

Behaviors	Description
AGGRESSIVE BEHAVIORS	
Aggression	Grab, hit, bite or slap
Threat	One or more of the following: open mouth stare, head bob, lunge
Chase	Quick, directed movement after another animal lasting > 3sec
Displacement	When another animal intentionally “takes the place” of another animal
OTHER BEHAVIORS	
Lipsmack ^a	Rapid lip movements with pursed or puckered lips
Crooktail	Animal demonstrates a stiff-legged strut and holds tail in a stiff “?”
Cage Shake	Animal vigorously shakes cage bars or slams body against the cage.
Present Groom	Rigid presentation of body part for grooming
Present Mount	Stiff, 4-point stance, tail up, rump orientated toward another animal
Scratch	Scratches own body
Yawn	Yawn

Note: Behaviors marked with ^a were also used to assess infant interest

Table 2

Mother-Infant Spacing Ethogram

Behaviors	Description
Duration Behaviors	
Physical Contact	Physical contact between infant and mother for > 3sec
Proximity	Infant within arm's reach of its mother for > 3sec
Nonsocial	Infant not in contact nor in arm's reach of its mother for > 3sec

Table 3

	Pre-birth Dyads					Post-birth Dyads				
	AMY MEAN	CON MEAN	HIP MEAN	Univariate ANOVA Lesion Effect	Post Hoc	AMY MEAN	CON MEAN	HIP MEAN	Univariate ANOVA Lesion Effect	Post Hoc
FEAR BEHAVIORS										
Avoid Initiate	0.525±0.389	0.219±0.063	0.125±0.153	($F_{(2,11)} = 3.340, p = 0.0735$)	-----	1.256±0.971	0.625±0.605	0.339±0.317	($F_{(2,11)} = 2.280, p = 0.1485$)	-----
Fear Grimace Initiate	3.675±3.929	1.406±1.709	1.375±1.183	($F_{(2,11)} = 1.214, p = 0.3338$)	-----	1.733±2.174	0.979±1.558	0.817±0.779	($F_{(2,11)} = 0.452, p = 0.6478$)	-----
Flee Initiate	0.025±0.056	0.031±0.063	0.075±0.112	($F_{(2,11)} = 0.538, p = 0.5987$)	-----	0.089±0.063	0.292±0.211	0.228±0.301	($F_{(2,11)} = 1.065, p = 0.3777$)	-----
Freeze Initiate	0.000±0.000	0.031±0.063	0.000±0.000	($F_{(2,11)} = 1.310, p = 0.3089$)	-----	0.011±0.025	0.007±0.014	0.022±0.023	($F_{(2,11)} = 0.611, p = 0.5602$)	-----
Scream Initiate	1.900±4.109	0.031±0.063	0.100±0.105	($F_{(2,11)} = 0.877, p = 0.4431$)	-----	0.428±0.818	0.167±0.177	0.178±0.185	($F_{(2,11)} = 0.396, p = 0.6822$)	-----
All Fear Behaviors Initiate	6.125±8.170	1.719±1.641	1.675±1.249	($F_{(2,11)} = 1.234, p = 0.3285$)	-----	3.517±3.331	2.069±2.138	1.583±1.369	($F_{(2,11)} = 0.839, p = 0.4581$)	-----
Social Interest Behaviors										
Approach Initiate	1.602±2.216	5.723±1.553	11.554±2.806	($F_{(2,11)} = 23.529, p = 0.0001$)	H>A($p=<.0001$) H>C($p=0.0031$) C>A($p=0.0219$)	6.278±4.301	7.021±4.263	13.583±10.421	($F_{(2,11)} = 1.540, p = 0.2572$)	-----
Contact Initiate	0.025±0.056	0.094±0.120	0.550±0.716	($F_{(2,11)} = 2.080, p = 0.1713$)	-----	0.272±0.323	1.653±2.304	5.356±5.907	($F_{(2,11)} = 2.415, p = 0.1351$)	-----
Follow Initiate	0.025±0.056	0.031±0.063	0.325±0.542	($F_{(2,11)} = 1.303, p = 0.3106$)		0.028±0.048	0.104±0.069	0.228±0.231	($F_{(2,11)} = 2.355, p = 0.1409$)	-----
Groom Initiate	0.000±0.000	0.063±0.125	0.325±0.727	($F_{(2,11)} = 0.745, p = 0.4971$)	-----	0.006±0.012	2.451±3.100	1.300±1.688	($F_{(2,11)} = 1.836, p = 0.2050$)	-----

	Pre-birth Dyads					Post-birth Dyads				
	AMY MEAN	CON MEAN	HIP MEAN	Univariate ANOVA Lesion Effect	Post Hoc	AMY MEAN	CON MEAN	HIP MEAN	Univariate ANOVA Lesion Effect	Post Hoc
FEAR BEHAVIORS										
PROXIMITY INITIATE	0.576±0.659	3.035±1.451	5.978±3.257	($F_{(2,11)} = 7.968, p = 0.0073$)	H>A($p = 0.0021$)	2.150±1.957	3.167±2.314	5.022±3.541	($F_{(2,11)} = 1.425, p = 0.2816$)	-----
INFANT INTEREST BEHAVIORS	AMY MEAN	CON MEAN	HIP MEAN	Univariate ANOVA Lesion Effect	Post Hoc	AMY MEAN	CON MEAN	HIP MEAN	Univariate ANOVA Lesion Effect	Post Hoc
AFFILIATIVE VOCALIZATIONS INITIATE	2.404±2.579	1.158±0.965	1.526±0.697	($F_{(2,11)} = 0.665, p = 0.5336$)	-----	4.444±3.615	18.028±6.881	12.589±3.657	($F_{(2,11)} = 9.591, p = 0.0039$)	C>A ($p = 0.0012$) H>A ($p = 0.0204$)
LIPSMACK INITIATE	1.352±1.637	0.470±0.694	0.552±0.548	($F_{(2,11)} = 0.932, p = 0.4228$)	-----	1.517±0.915	7.333±3.635	5.506±3.068	($F_{(2,11)} = 5.561, p = 0.0214$)	C>A ($p = 0.0084$) H>A ($p = 0.0399$)

Average number (frequency) of behaviors ± SD is shown for Pre-birth Dyads and Post-birth Dyads per amygdala-lesioned subjects (AMY), hippocampus-lesioned subjects (HIP), and sham-operated control subjects (CON).

Table 4

	Pre-birth Dyads			Block 1 of Post-birth Dyads		
	AMY MEAN	CON MEAN	HIP MEAN	AMY MEAN	CON MEAN	HIP MEAN
FEAR BEHAVIORS						
AVOID INITIATE	0.525±0.389	0.219±0.063	0.125±0.153	0.316±0.415	0.438±0.321	0.366±0.268
FEAR GRIMACE INITIATE	3.675±3.929	1.406±1.709	1.375±1.183	2.050±2.087	0.688±0.830	0.982±1.077
FLEE INITIATE	0.025±0.056	0.031±0.063	0.075±0.112	0.032±0.044	0.273±0.230	0.266±0.360
FREEZE INITIATE	0.000±0.000	0.031±0.063	0.000±0.000	0.000±0.000	0.000±0.000	0.033±0.075
SCREAM INITIATE	1.900±4.109	0.031±0.063	0.100±0.105	0.732±1.454	0.250±0.247	0.132±0.181
ALL FEAR BEHAVIORS INITIATE	6.125±8.170	1.719±1.641	1.675±0.1249	3.130±3.918	1.648±1.258	1.779±1.427
SOCIAL INTEREST BEHAVIORS						
APPROACH INITIATE	1.602±2.216	5.723±1.553	11.554±2.806	6.950±7.367	5.958±2.690	17.250±11.981
PROXIMITY INITIATE	0.576±0.659	3.035±1.451	5.978±3.257	2.382±2.043	2.813±1.770	6.982±4.281
INFANT INTEREST BEHAVIORS						
AFFILIATIVE VOCALIZATIONS INITIATE	2.404±2.579	1.158±0.965	1.526±0.697	6.414±5.894	13.270±3.975	13.900±4.853
LIPSMACK INITIATE	1.352±1.637	0.470±0.694	0.552±0.548	1.867±1.446	4.229±2.925	4.117±1.966

Average number (frequency) of behaviors $\pm$ SD is shown for Pre-birth Dyads and for Block 1 of Post-birth Dyads per amygdala-lesioned subjects (AMY), hippocampus-lesioned subjects (HIP), and sham-operated control subjects (CON).